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Clinical Presentation of Griscelli Syndrome Type 2 and Spectrum of *RAB27A* Mutations

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Background. Griscelli syndrome type 2 (GS2) is an autosomal-recessive immunodeficiency caused by mutations in *RAB27A*, clinically characterized by partial albinism and haemophagocytic lymphohistiocytosis (HLH). We evaluated the frequency of *RAB27A* mutations in 21 unrelated patients with haemophagocytic syndromes without mutations in familial HLH (FHL) causing genes or an established diagnosis of GS2. In addition, we report three patients with known GS2. Moreover, neurological involvement and *RAB27A* mutations in previously published patients with genetically verified GS2 are reviewed. ***Procedure.*** Mutation analysis of *RAB27A* was performed by direct DNA sequencing. NK cell activity was evaluated and microscopy of the hair was performed to confirm the diagnosis. ***Results.*** *RAB27A* mutations were found in 1 of the 21 families. This Swedish family had three affected children with heterozygous compound mutations consisting of a novel splice error mutation, [c.239G>C], and a nonsense mutation, [c.550C>T], p.R184X. The three additional children all carried homozygous *RAB27A* mutations, one of which is a novel splice error mutation, [c.240-2A>C]. Of note, five of the six patients displayed neurological symptoms, while three out of six patients displayed NK cell activity within normal reference values, albeit low. A literature review revealed that 67% of GS2 patients have been reported with neurological manifestations. ***Conclusions.*** Identification of *RAB27A* mutations can facilitate prompt diagnosis and treatment, and aid genetic counselling and prenatal diagnosis. Since five of six patients studied herein initially were diagnosed as having FHL, we conclude that the diagnosis of GS2 may be overlooked, particularly in fair-haired patients with haemophagocytic syndromes. Pediatr Blood Cancer 2010;54:563–572. © 2009 Wiley-Liss, Inc.

Key words: albinism; Griscelli syndrome type 2; haemophagocytic lymphohistiocytosis; haemophagocytic syndrome; *RAB27A*

INTRODUCTION

Haemophagocytic lymphohistiocytosis (HLH) is characterised by fever, cytopenia, hepatosplenomegaly and hypercytokinaemia [1–3]. Histopathological findings include an accumulation of lymphocytes as well as macrophages in affected tissues, particularly in the spleen, lymph nodes, bone marrow, liver and cerebrospinal fluid, sometimes with haemophagocytosis [2,4]. HLH can be divided into primary, familial forms [1] and secondary forms [5]. The diagnosis requires fulfilment of at least five of the eight diagnostic criteria, established by the Histiocyte Society [2]. Alternatively, identification of mutations in one of the genes associated with development of HLH may provide the diagnosis [2]. A characteristic finding in patients with primary HLH is defective lymphocyte cytotoxic function, mediated by natural killer (NK) cells and cytotoxic T cells (CTL) [6–8]. Typically, primary HLH is fatal unless treated by haematopoietic stem cell transplantation (HSCT).

There are currently three autosomal-recessive gene defects that have been uniquely associated with familial HLH (FHL), namely *PRF1* encoding perforin [9,10], *UNC13D* encoding Munc13-4 [11,12] and *STX11* encoding syntaxin-11 [13,14]. In addition, X-linked lymphoproliferative syndrome (XLP), characterised by life-threatening Epstein–Barr virus (EBV) infection, is frequently associated with development of a haemophagocytic syndrome [15], and is caused by mutations in *SH2D1A* or *XIAP* [16,17]. Another group of autosomal-recessive gene defects associated with haemophagocytic syndromes distinctively present with a partial albinism and include Griscelli syndrome type 2 (GS2) and Chediak–Higashi syndrome type 1 (CHS1), caused by mutations in *RAB27A* and *LYST*, respectively [18,19]. Together, the proteins encoded by these genes have all been implicated in lymphocyte cytotoxicity.

Griscelli syndrome (GS) was first described by Griscelli *et al.* in 1978 [20]. GS and CHS1 both manifest partial albinism, sometimes including eyebrows and eyelashes [20]. GS can be distinguished from CHS1 by the lack of leukocyte cytoplasmic giant-granules [20]. Furthermore, GS is subdivided into three genetically distinct entities [18,21,22]. In addition to GS2, GS1 is caused by mutations

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in *MYO5A* (encoding myosin-Va) and presents with neurological manifestations and partial albinism [22], whereas GS3 is caused by mutations in *MLPH* (encoding melanophilin) and the phenotype is restricted to partial albinism [21]. Among the GS subtypes, only GS2 is associated with the development of a haemophagocytic syndrome.

RAB27A encodes Rab27a, which is a member of the small GTPase family of proteins involved in vesicular fusion and trafficking [18]. GTP-bound Rab27a binds Munc13-4 [23]. Both proteins are required for perforin-dependent lymphocyte cytotoxicity, which occurs by fusion of secretory lysosome with the plasma membrane [11,18]. In melanocytes, Rab27a, melanophilin and myosin-Va are all required for distribution of pigment-containing melanosomes, which constitute a specialised lysosomal compartment related to cytotoxic lymphocyte secretory lysosomes [21]. However, unlike Rab27a, melanophilin and myosin-Va are not expressed in cytotoxic lymphocytes [24].

Notably, the albinism in GS2 may be subtle and there is a risk that children clinically diagnosed with FHL, but without a verified molecular diagnosis, may instead carry mutations in *RAB27A*. Therefore, one aim of the current study was to determine the frequency of *RAB27A* mutations in a cohort of patients with well-defined primary HLH, having excluded patients with mutations in known FHL genes and patients with a known clinical diagnosis of GS2. We also aimed to expand the spectrum and describe the clinical presentation of *RAB27A* mutations in patients previously clinically diagnosed with GS2. In addition, we present the location of *RAB27A* mutations, relative to exon structure and protein domains, in all GS2 patients with verified mutations published in the English literature (up to March 1, 2009) and review the reported neurological manifestations in these patients.

PATIENTS AND METHODS

Patients

Cohort 1: To determine the frequency of *RAB27A* mutations in children with HLH not known to have GS2, we studied a cohort of 23 individuals from 21 families in which biallelic mutations in *PRF1*, *STX11* and *UNC13D* had been excluded by direct sequencing [10,14,25]. All individuals studied fulfilled the diagnostic criteria for HLH according to the HLH-2004 protocol [2]. In addition, the patients had either undergone HSCT or had a survival of less than 1 year without having a HSCT. Six patients had a familial disease, and five were born to consanguineous parents. Thirteen families were of Nordic origin, five originated from Turkey and three were from other parts of Europe. *Cohort 2*: To expand the mutation spectrum of *RAB27A*, we analysed three additional patients with a clinical diagnosis of GS2, from which DNA was available. One of these patients has been described by zur Stadt *et al.* [26]. DNA from 96 healthy adult blood donors was used as a control. The studies were approved by the ethics committee at Karolinska Institutet. Written parental consent was obtained to publish the photographs of patients.

Methods

Sequencing analyses. Genomic DNA was isolated from peripheral blood or cultured fibroblasts according to standard procedures. In some cases, screening for mutations of parental DNA was performed when the amount of material from the children was limited. Specific primers were used for amplification of the coding sequence (exons 2–6) of *RAB27A*, direct sequencing was performed on ABI 3730 Genetic Analyzers (Applied Biosystems, Foster City, CA), and data were analysed using SeqScape (Applied Biosystems), or, alternatively, by manual analysis. As a reference sequence, NCBI Accession NM_004580 was used. Primers, polymerase chain reaction (PCR) conditions and sequencing reaction conditions are available upon request.

Light and transmission electron microscopy of hair samples. For light microscopy, small pieces of hair from the patients were cut and placed on a glass slide. The slides were mounted with Eukitt (O. Kindler GmbH & Co., Freiburg, Germany), and specimens were examined in an Eclipse C1000 microscope (Nikon, Tokyo, Japan). Images were obtained using a digital camera (DXM 1200F, Nikon) operating with ACT-1 software (Nikon).

For transmission electron microscopy, small pieces of hair were cut with a razor and fixed. Ultrathin sections (approximately 40–50 nm) were cut and contrasted with uranyl acetate followed by lead citrate and examined in a Tecnai 10 (Phillips, FEI, Eindhoven, the Netherlands) at 80 kV. Digital images were taken with a MegaView III digital camera (Soft Imaging System GmbH, Münster, Germany).

Measurements of NK cell cytotoxic activity. NK cell activity was assessed as previously described [8]. In brief, ^{51}Cr-labelled K562 target cells were incubated with peripheral blood lymphocytes (PBL) as effector cells for 4 hr. Supernatants were analysed with a γ-counter. A value of 10 lytic units (LU) or less at 25% lysis is considered pathological [2], and here we also define a value from 10 to 25 LU as low.

RESULTS

Characterization of *RAB27A* mutations

Cohort 1: Among the individuals previously screened and found negative for mutations in the FHL-associated genes *PRF1*, *STX11* and *UNC13D*, DNA from 21 families (23 individuals) was analysed for mutations in *RAB27A*. Among these families, mutations in *RAB27A* were found in one Swedish family (Family A, three individuals) (Table I). The three children in Family A harboured heterozygous compound mutations, including a novel mutation [c.239G>C] corresponding to the last nucleotide in exon 3 (Fig. 1A). Such a substitution in the consensus splice donor sequence is predicted to interfere with mRNA splicing [27]. The second mutation in Family A is a nonsense mutation [c.550C>T] with a predicted premature stop codon, p.Arg184X in exon 6, and has previously been described by Menasche et al. [18]. The fair hair of one of the twin sisters in Family A, not considered abnormal by treating physicians, is depicted (Fig. 2A). However, corroborating the genetic analysis, microscopic examination of hair from the twins revealed typical irregular accumulation of pigment in hair shafts (Fig. 2B), as compared to that from a healthy fair-haired control (Fig. 2C) [20].

Cohort 2: In addition to cohort 1, homozygous mutations in *RAB27A* were identified in three children with an initial clinical diagnosis of GS2 (Families B–D; Table I). Patient B:1 is homozygous for the p.Arg184X mutation as previously reported by Trottestam et al. [28]. Patient C:1 harboured a novel, homozygous *RAB27A* mutation [c.240-2A>C]. This mutation

TABLE I. Familial Data, Mutation Data and Clinical Findings in Patients With Biallelic *RAB27A* Gene Mutations

	Family A			Family B	Family C	Family D
Patient	A:1	A:2	A:3	B:1	C:1	D:1
Origin	Sweden	Sweden	Sweden	Denmark	Pakistan	Germany
Familial disease	Yes	Yes	Yes	No	No	Yes
Consanguinity	No	No	No	No	Yes	Yes
Sex	Male	Female	Female	Male	Female	Female
Mutation	[c.239G>C], [c.550C>T]	[c.239G>C], [c.550C>T]	[c.239G>C], [c.550C>T]	[c.550C>T]	[c.240-2A>C]	[c.148_149delinsC]
Protein alteration	Splice error, p.Arg184X, compound	Splice error, p.Arg184X, compound	Splice error, p.Arg184X, compound	p.Arg184X, homozygous	Splice error, homozygous	p.Arg50GlnfsX35, homozygous
Age at diagnosis of HLH	217 days	55 days	62 days	107 days	13 years	13 months
Age at diagnosis of GS2	7 years	1.5 years	1.5 years	1.5 years	13 years	8 years
Fever	Yes	Yes	Yes	Yes	Yes	Yes
Splenomegaly	Yes	Yes	Yes	Yes	Yes	Yes
Hepatomegaly	Yes	Yes	Yes	No	No	Yes
Hb (g/L)	82	46	62	85	81	Transfusion dependent
Neutrophils (10^9/L)	1.3	0.74	0.33	0.63	2.0	1.3
Platelets (10^9/L)	72	10	85	52	53	37
Triglycerides (mmol/L)	7.6	3.6	3.3	4.1	n.d.	13.5
Fibrinogen (g/L)	1.5	n.d.	n.d.	3.1	1.9	0.81
Ferritin (μg/L)	512	2,293	1,596	3,145	940	n.d.
Haemophagocytosis	No	n.d.	n.d.	No	No	Yes
sCD25 (U/ml)	10,890	n.d.	n.d.	n.d.	n.d.	n.d.
NK cell activity[a]	Pathological	Low	Pathological	Low	Low	Pathological
Neurological manifestations[b]	Yes	Yes	No	Yes	Yes	Yes
Hair pathology[c]	Yes	Yes	Yes	Yes	Yes	Yes
Treatment protocol	HLH-94, ITMTX	HLH-94	HLH-94	HLH-94, ITMTX, ITPred	HLH-2004	VP16, pred, CSA, ITMTX
Remission at 2 months	No	No	No	No	No	n.d.
Age at HSCT	310 days	141 days	141 days	15 months	13 years	22 months
Outcome	Alive	Alive	Alive	Alive	Alive	Alive

ITMTX, intrathecal methotrexate; ITPred, intrathecal prednisolone; Pred, prednisolone; CSA, cyclosporin A; n.d., no data available. [a]Pathological: 10 lytic units or less; low: from 10 to 25 lytic units; [b]Neurological manifestations reported at some point during the course of the disease; [c]Microscopic findings of hair consistent with GS2.

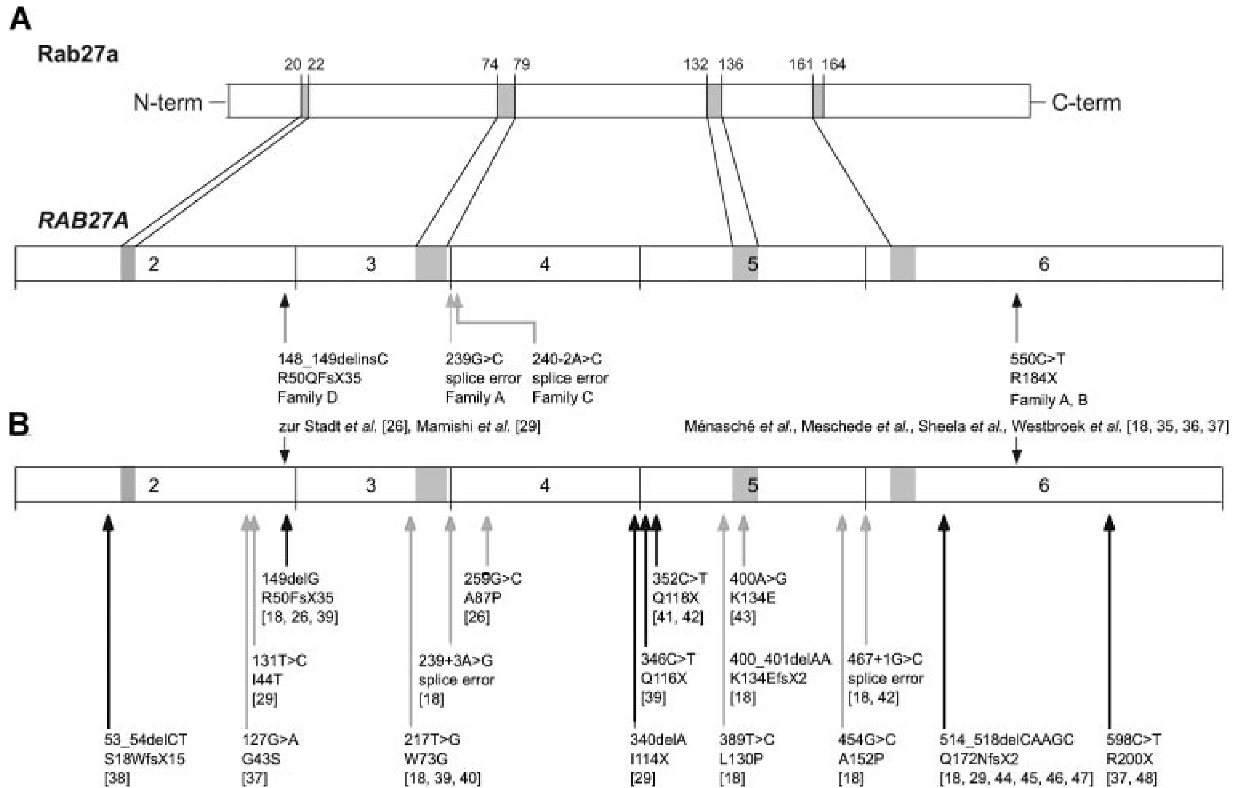

Fig. 1. Location of the mutations in *RAB27A* in patients with GS2 described in this report and in previous publications relative to exon structure and protein domains. **A**: Sites of mutations, described in this report. **B**: Sites of previously reported mutations [18,26,29,35–48]. Exon borders and the highly conserved GTP-binding domains (grey box) are indicated. The shades of arrows denote different types of mutations: disruptive nonsense and small deletion mutations (black); missense mutations and splice-site mutations (grey). In addition, three gross deletions have been reported, namely deletion of exons 2–5 [26], exons 3–4 [18] and promoter–exon 5 [49].

affects the conserved splice acceptor site in intron 3, with predicted splice error [27]. Patient D:1, previously reported by zur Stadt et al. [26], carries a homozygous mutation described in an unrelated family by Mamishi et al. [29], [c.148_149delinsC] that is predicted to result in a frameshift and premature stop codon [p.Arg50GlnfsX35].

The novel *RAB27A* mutations identified in Families A and C (Table I, Fig. 1A) were not found among 96 sequenced healthy adult blood donors (192 alleles). Figure 1A indicates the position of *RAB27A* mutations in our GS2 cohort, relative to previously reported GS2 patients summarised in Figure 1B.

Notably, in five of the six patients investigated for *RAB27A* mutations (cohorts 1 and 2), the clinical diagnosis of GS2 was delayed. All patients except C:1 were initially diagnosed and treated as having FHL.

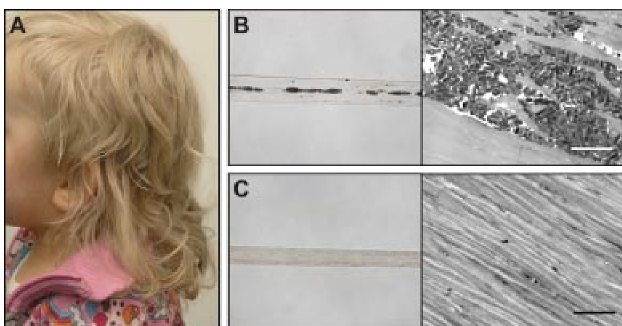

Fig. 2. Hair structure in patients with Griscelli syndrome type 2. **A**: Patient A:2 after haematopoietic stem cell transplantation. The typical silvery hair is not always prominent, and easily overlooked, particularly in fair-haired individuals with haemophagocytic syndromes. **B**: Characteristic irregular accumulation (clumping) of melanin is seen in hair shafts from a female patient with GS2 (Family A) in contrast to the control hair of a healthy control (**C**). The difference is evident both upon light microscopic (**left panels**) and electron microscopic examination (**right panels**) (bar 2 µm).

Evaluation of NK cell activity

Decreased NK cell activity, evaluated in all patients in this study, is one of eight diagnostic criteria for HLH, typically assessed by quantifying lysis of K562 target cells. With resting PBMC as effector cells, a value of 10 LU or less is indicative of HLH [2]. Remarkably, NK cell activity at clinical presentation was determined to 22 LU in patient A:2. Upon re-evaluation after treatment, this had decreased to 1 LU. Patient A:3 had an NK cell activity of 5 LU at clinical presentation, that decreased to 0 LU after treatment. NK cell activity in Patient B:1 was 20 LU and in patient C:1 11 LU, both higher than the HLH criterion value, while NK cell activity was reported absent in patients A:1 and D:1. In conclusion, in three out of

six patients NK cell activity was demonstrated to be low, yet above 10 LU (the limit set as pathological for diagnosis of HLH) (Table I).

Clinical presentations

Family A comprises one affected male and two affected, younger, identical twin sisters (Tables I and II). The male patient was initially referred at the age of 79 days. Prior to being diagnosed with HLH, he developed neurological alterations (Table III). Magnetic resonance imaging (MRI) revealed abnormalities in the brain stem, thalamus and cerebellum, and analysis of the cerebrospinal fluid (CSF) showed pleocytosis (36×10^6 cells/L). One of the twins (A:2) had convulsions during the pre-HSCT immunochemotherapy, and MRI revealed a picture consistent with an infarction in arteria cerebri media. Follow-up MRI showed regression of the identified abnormalities. They were all treated with the HLH-94 protocol including HSCT with cord blood [30]. Whereas neurological sequelae (including left-sided facial palsy) were reported at the male patient's last follow-up, the twin sisters have no current signs of neurological involvement.

Patient B:1 was first admitted at 5 weeks of age with moderate pancytopenia and pneumonia. He recovered while receiving antibiotics and supportive care, but was readmitted at the age of 3 months with symptoms of HLH (Table I). The CSF cell count (19×10^6 cells/L) and protein level (0.65 g/L) were both elevated. He received treatment with HLH-94 and improved clinically. The pleocytosis disappeared without intrathecal (IT) therapy, but during continuation therapy a right-sided paresis occurred. Although his CSF was normal, MRI revealed diffuse changes of white matter in both hemispheres extending into the basal ganglia and medulla oblongata. The neurological status improved partially, but he remained hemiparetic. At the age of 5 years, he still suffers from impaired vision, retarded speech and a moderate right-sided hemiparesis. The patient has always, in contrast to other family members, had completely white hair (Fig. 3). However, at the time for onset of HLH, GS2 was not suspected. The suspicion of GS2 was raised at the time of HSCT and was confirmed by microscopic examination of the hair.

Patient C:1 was admitted at the age of 6 years because of fever and hepatosplenomegaly. Thereafter, she was not hospitalised until the age of 13, when she developed HLH secondary to an EBV infection. MRI revealed cerebral, cerebellar and central atrophy. She was diagnosed with GS2 on the basis of microscopy of the hair. She suffered from severe generalised muscle impairment due to pronounced myositis that was verified by biopsy and which caused progressive weakness. Her cognitive function was also impaired. After the HSCT, her mental function improved markedly.

Patient D:1 developed symptoms of HLH at the age of 13 months (Table I). Immunochemotherapy was initiated, and after marked improvement, administration of prednisolone and etoposide (VP16) was stopped. However, while on cyclosporin A (CSA), the patient relapsed, presumably in association with an EBV infection, and had convulsions. Treatment with prednisolone, VP16 and IT MTX was initiated again, and subsequently HSCT was performed. Besides a short stature, there are no long-term sequelae. The silvery strands of the hair have been very discrete and microscopy was only performed at the age of 8 years, and the homozygous *RAB27A* mutations were detected at the age of 12 years.

The clinical presentation of neurological manifestations in five out of six patients prompted us to perform an evaluation of such symptoms and other clinical features in GS2 patients reported in the literature (Tables II and III; 18 patients were omitted due to a paucity of clinical data). Among the 43 patients described with verified *RAB27A* mutations, 29 patients (67%) were reported with clinical neurological manifestations during the course of the disease (Table III). The corresponding figures for neurological manifestations at diagnosis were 21/38 patients (55%). Among the neurological symptoms described (listed in Table III), seizures and cranial nerve palsy were the most commonly reported. Other reported symptoms were paresis, delayed psychomotor development, disturbances in the levels of consciousness, ataxia and hypotonia. The median age at onset of HLH was 6.5 months ($n = 30$, mean 24.0 months; range 1.8 months to 13.5 years) and median age at diagnosis 6.1 months ($n = 18$, mean 31.5 months; range 1.8 months to 13.5 years). Patients with reported onset/diagnosis at 12 months of age or above had a significantly higher frequency of neurological manifestations compared to those with reported onset/diagnosis below 12 months of age ($n = 38$, $P = 0.005$, Fisher's exact test two-sided). In addition, 36% of the GS2 patients with an affected, previously diagnosed relative were reported to have neurological manifestations compared to 72% of those without ($n = 29$, $P = 0.12$).

DISCUSSION

Our study highlights the importance of considering the diagnosis GS2 among patients with haemophagocytic syndromes, particularly for fair-haired individuals in whom partial albinism may be overlooked. Among the individuals previously screened and found negative for mutations in the FHL-associated genes *PRF1*, *STX11* and *UNC13D*, biallelic *RAB27A* mutations were identified in 1 of the 21 families. Two of the *RAB27A* mutations presented here are novel. We also report that neurological manifestations, typically associated with GS1 [21,22], were present in five out of six patients, belonging to four families, with verified mutations in *RAB27A*.

Clinically, patients with GS2 can have a presentation that resembles FHL. In addition, GS2 patients typically display partial albinism [20]. However, since the silvery colouring of the hair may be subtle, as in patients reported here, it is also important to consider the diagnosis of GS2 in patients without obvious signs of silvery hair. Our study revealed bialleic mutations in *RAB27A* in three children (from one family) previously diagnosed with FHL. Similarly, two out of three of the additional GS2 patients studied herein were initially diagnosed with FHL, further highlighting the importance of considering GS2 in patients with HLH. Early diagnosis is important for several reasons. First, rapid diagnosis facilitates prompt initiation of appropriate treatment, likely reducing the risk of developing life-long neurological sequelae. In our study, only patients with previously affected siblings were without sequelae at follow-up, thus reiterating the importance of early diagnosis and treatment. Second, since treatment includes HSCT [2], which is associated with fatal complications, a molecular diagnosis is of value for supporting the decision to perform HSCT. Finally, identification of a mutation can provide an opportunity for genetic counselling and prenatal diagnosis.

The age of diagnosis of HLH in the current study varied markedly, from less than 2 months (patient A:2) to more than 13 years (patient C:1) (median 5 months) (Table I). In a previous study, Klein et al. [31] reported a median age for developing a haemophagocytic syndrome in GS2 patients of 3.5 years ($n = 7$). In

TABLE II. Review of Clinical Data of Published Patients With *RAB27A* Mutations

Pat. ID [Ref.]	Sex	Protein alteration	Age at onset/ diagnosis	Cons./Fam	Previously diagnosed relative	Treatment	HSCT	Outcome/ follow-up (years)	Fever	Splenome-galy	Bicyto-penia	Triglycerides ↑ /Fibrinogen ↓	Ferritin ↑	sCD25 ↑	Haemo-phago-cytosis	Defective NK cell activity
A:1[a]	M	Splice error, R184X	2.5/7m	No/yes	No	HLH-94, ITMTX	Yes	Alive/+7	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
A:2[a]	F	Splice error, R184X	2/2m	No/yes	Yes	HLH-94	Yes	Alive/+2	Yes	Yes	Yes	Yes	Yes	—	—	Borderline
A:3[a]	F	Splice error, R184X	2/2m	No/yes	Yes	HLH-94	Yes	Alive/+2	Yes	Yes	Yes	Yes	Yes	—	—	Yes
B:1[a]	M	R184X	3/3.5m	No/no	No	HLH-94, ITMTX, ITPred	Yes	Alive/+5	Yes	Yes	Yes	Yes	Yes	—	No	Borderline
C:1[a]	F	Splice error	13/13y	Yes/no	No	HLH-2004	Yes	Alive	Yes	Yes	Yes	No	Yes	—	No	Borderline
D:1[a]	F	R50QfsX35	13/13m	Yes/yes	Yes	Other	Yes	Alive	Yes	Yes	Yes	Yes	—	—	Yes	Yes
P1 [29]	M	Q172NfsX2	72m/—	Yes/yes	No	—	No	Dead	Yes	Yes	No	Yes	Yes	—	Yes	—
P2 [29]	F	Q172NfsX2	3m/—	Yes/yes	Yes	—	Planned	Alive	Yes	Yes	No	No	No	—	No	—
P3 [29]	M	Q172NfsX2	8m/—	Yes/yes	—	—	No	Dead	Yes	Yes	Yes	Yes	Yes	—	Yes	—
P4 [29]	M	Q172NfsX2	5m/—	Yes/yes	—	HLH-94	Planned	Alive	Yes	Yes	Yes	Yes	Yes	—	Yes	—
P5 [29]	M	Q172NfsX2	36m/—	Yes/no	No	—	No	Dead	Yes	Yes	Yes	—	—	—	Yes	—
P6 [29]	F	I114X	2m/—	Yes/yes	Yes	Other	Planned	Alive	Yes	Yes	Yes	Yes	Yes	—	No	—
P7 [29]	M	I44T	20m/—	Yes/no	No	Other	No	Dead	Yes	Yes	Yes	Yes	—	—	Yes	—
P8 [29]	M	R50QfsX35	24m/—	Yes/no	No	HLH-94	—	Alive/+3	Yes	Yes	Yes	Yes	Yes	—	No	—
P9 [29]	M	Q172NfsX2	6m/—	Yes/no	No	HLH-94	No	Dead	Yes	Yes	Yes	Yes	Yes	—	Yes	—
[35]	M	R184X	36m/—	No/yes	No	—	No	Dead	—	—	—	—	—	—	—	—
[35]	M	R184X	45m/—	No/yes	Yes	Other	No	Dead	Yes	Yes	Yes	—	—	—	—	—
[36]	M	R184X	[b]	Yes/yes	Yes	—	Considered	Alive	No	—	—	—	—	—	—	—
P1 [37]	F	R184X, R200X	[b]	No/yes	Yes	—	Yes	Alive/+4.5	—	—	—	—	—	—	—	—
P2 [37]	M	G43S	36/72m	Yes/no	No	HLH-94	Yes	Alive/+5	Yes	Yes	Yes	—	Yes	—	—	Yes
[38]	F	S18WfsX15	8/11y	Yes/yes	Yes	Other	No	Dead	Yes	Yes	Yes	No	—	—	No	—
1 [39]	M	R50KfsX35	—/5m	Yes/—	—	Modified HLH-94	No	Dead	Yes	Yes	Yes	—	—	—	—	—
2 [39]	M	R50KfsX35	—/9m	Yes/—	—	Other	No	Dead	Yes	Yes	No	—	—	—	—	—
3 [39]	M	R50KfsX35	—/4m	Yes/—	—	HLH-94	No	Dead	Yes	Yes	Yes	—	—	—	—	—
4 [39]	F	R50KfsX35	—/3m	Yes/—	—	Other	No	Dead	Yes	Yes	Yes	—	—	—	—	—
5 [39]	F	R50KfsX35	—/2m	Yes/—	—	HLH-94	Yes	Dead	Yes	Yes	Yes	—	—	—	—	—
6 [39]	F	W73G	—/48m	Yes/—	—	HLH-94	Planned	Alive/+1	Yes	Yes	No	—	—	—	—	—
7 [39]	F	W73G	—/48m	Yes/—	—	HLH-94	Yes	Alive/+5	Yes	Yes	Yes	—	—	—	—	—
8 [39]	F	Q116X	—/4y	Yes/—	—	HLH-94	No	Dead	Yes	Yes	Yes	—	—	—	—	—
[41]	F	Q118X	4m/—	Yes/no	No	Other	Planned	Alive	Yes	Yes	—	Yes	Yes	—	Yes	Yes
1 [42]	M	Q118X splice error	5m/—	No/no	No	ATG	No	Dead	Yes	Yes	Yes	—	—	—	Yes	Yes
[43]	M	K134E	69m/—	Yes/yes	No	Other	No	Dead	Yes	No	Yes	—	—	—	No	—
[44]	F	Q172NfsX2	3/3m	Yes/no	No	ATG, ITMTX	Yes	Alive/+2.5	Yes	Yes	—	—	—	—	Yes	—

[45]	M	Q172NfsX2	5.5m/—	Yes/no	No	HLH-94	Yes	Alive	Yes	Yes	Yes	Yes	Yes	—	Yes	Yes
[46]	M	Q172NfsX2	3m/—	Yes/yes	Yes	HLH-94	No	Dead	Yes	Yes	Yes	No	No	—	Yes	—
[46]	M	Q172NfsX2	3m/—	Yes/Yes	Yes	HLH-94	No	Dead	Yes	Yes	Yes	Yes	No	—	Yes	—
[47]	M	Q172NfsX2	3/3m	No/no	No	Dexa, VP16, CSA	Planned	Alive/1.5	Yes	Yes	Yes	Yes	—	—	Yes	—
[48]	F	R200X	32m/—	Yes/no	No	—	—	—	No	No	No	—	—	—	Yes	—
P1 [49]	F	Del pro-ex5	—	Yes/yes	—	—	No	Dead	—	Yes	—	—	—	—	—	—
P2 [49]	F	Del pro-ex5	—	Yes/yes	—	—	No	Dead	—	—	—	—	—	—	—	—
P3 [49]	F	Del pro-ex5	—	Yes/yes	—	—	No	Dead	—	—	—	—	—	—	—	—
P4 [49]	M	Del pro-ex5	1y/—	Yes/yes	—	—	No	Dead	Yes	Yes	Yes	—	—	—	—	—
[50]	M	—	7m/—	Yes/no	No	ATG, ITMTX	No	Dead	Yes	Yes	Yes	Yes	—	—	Yes	—
Total				79%/60%	11/29, 38%		12/41, 29%		36/38, 95%	36/38, 95%	30/35, 86%	17/21, 81%	14/17, 82%	1/1	16/24, 67%	

ATG, anti-thymocyte globulin-based treatment; ITMTX, intrathecal methotrexate; ITPred, intrathecal prednisolone; Dexa, dexamethasone; VP16, etoposide; CSA, cyclosporin A; —, no data; Cons., reported parental consanguinity; Fam, reported familial disease; m, months; y, years. [a]Patient described in the current study; [b]Had not developed any clinical disease at the time of the initial publication.

our comprehensive review of patients reported with *RAB27A* mutations the median age at onset was 6.5 months and median age at diagnosis 6.1 months. The median age at diagnosis for FHL patients with *PRF1*, *UNC13D* and *STX11* mutations has been reported to 2.3, 6.2 and 14.4 months, respectively [32].

We report neurological manifestations during the course of the disease in all four families. Our review of patients reported with *RAB27A* mutations revealed neurological manifestations in 55% of patients at diagnosis and in 67% of patients during the course of the disease. Together, findings highlight the fact that not only GS1 but also GS2 is frequently associated with neurological impairment. By comparison, Horne et al. [33] reported a lower frequency (37%) of clinical neurological abnormalities at diagnosis in children with HLH. The fact that a third of GS2 patients have no reported neurological manifestations, that patients with reported onset/diagnosis at 12 months of age or above were shown to have higher frequency of neurological manifestations, and that knowledge of familiar disease may reduce the incidence of neurological sequelae in affected younger siblings suggest that neurological manifestations in GS2 are secondary to HLH, rather than a direct consequence of possible Rab27a dysfunction in the central nervous system. In line with this, Pachlopnik Schmid et al. [34] recently argued, in a single-centre study of 10 patients, that neurological symptoms in GS2 patients are secondary to HLH. Patient C:1 improved after HSCT and patients A:3 and D:1 have no neurological manifestations after HSCT, supporting that not only prompt initiation of therapy but also prompt HSCT is valuable in order to reduce late neurological sequelae in GS2.

We identified two novel *RAB27A* mutations in our patients: [c.239G>C] and [c.240-2A>C], both of which are predicted to result in splice errors. These novel alterations were not found in healthy blood donors suggesting that the mutations are pathogenic. These conclusions were supported by the characteristic microscopic findings of irregularly distributed accumulations of melanin, mostly located in the medullar zone of the hair shaft [20]. Thus, typical microscopic findings are a simple and useful complement to the molecular diagnosis and should be routinely performed in cases where FHL is suspected. Furthermore, it is noteworthy that three out of six evaluated patients demonstrated NK cell activity somewhat above 10 LU. Therefore, a value above this level does not exclude GS2.

In conclusion, this study highlights the importance of considering GS2 in all children presenting with a haemophagocytic syndrome, and to be aware of subtle signs of partial albinism, not least in fair-haired children, and to study hair microscopy more frequently. A molecular diagnosis is helpful for genetic counselling and prenatal diagnosis. An early diagnosis may also be valuable for the prompt initiation of treatment and the reduction of late sequelae, in particular neurological sequelae, which are not infrequent in patients with *RAB27A* mutations.

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TABLE III. Review of Central Nervous System Involvement in Published Patients With *RAB27A* Mutations

Ref.	Sex	Protein alteration	Age at onset/diagnosis	Cons./Fam	Previously diagnosed relative	Neuro at diag.	Neuro during course	Treatment	HSCT	Specified neurological alterations	Pathological CSF	Pathological MRI
A:1[a]	M	Splice error, R184X	2.5/7m	No/yes	No	Yes	Yes	HLH-94, ITMTX	Yes	Seizures, nystagmus, eye adduction deficiency, left-sided facial palsy	Yes	Yes
A:2[a]	F	Splice error, R184X	2/2m	No/yes	Yes	No	Yes	HLH-94	Yes	Seizures	—	Yes
A:3[a]	F	Splice error, R184X	2/2m	No/yes	Yes	No	No	HLH-94	Yes		—	—
B:1[a]	M	R184X	3/3.5m	No/no	No	No	Yes	HLH-94, ITMTX, ITPred	Yes	Hemiparesis, impaired speech and vision	Yes	Yes
C:1[a]	F	Splice error	13/13y	Yes/no	No	Yes	Yes	HLH-2004	Yes	Impaired cognitive function	—	Yes
D:1[a]	F	R50QfsX35	—/13m	Yes/yes	Yes	No	Yes	Other	Yes	Seizures	Yes	—
P1 [29]	M	Q172NfsX2	72m/—	Yes/yes	No	Yes	Yes	—	No	Right-sided hemiplegia, progressive encephalopathy, seizures and coma	—	Yes
P2 [29]	F	Q172NfsX2	3m/—	Yes/yes	Yes	No	No	—	Planned		—	—
P3 [29]	M	Q172NfsX2	8m/—	Yes/yes	—	No	No	—	No		—	—
P4 [29]	M	Q172NfsX2	5m/—	Yes/yes	—	No	No	HLH-94	Planned		—	—
P5 [29]	M	Q172NfsX2	36m/—	Yes/no	No	Yes	Yes	—	No	Bilateral esoptropia	—	—
P6 [29]	F	I114X	2m/—	Yes/yes	Yes	No	No	Other	Planned		—	—
P7 [29]	M	I44T	20m/—	Yes/no	No	Yes	Yes	Other	No	Inability to walk	—	Yes
P8 [29]	M	R50QfsX35	24m/—	Yes/no	No	No	No	HLH-94	—		—	—
P9 [29]	M	Q172NfsX2	6m/—	Yes/no	No	Yes	Yes	HLH-94	No	Exotropia of the right eye	—	—
[35]	M	R184X	36m/—	No/yes	No	Yes	Yes	—	No	Symptoms of encephalitis	—	Yes
[35]	M	R184X	45m/—	No/yes	Yes	Yes	Yes	Other	No	Ataxia, tetraparesis, coma	Yes	Yes
[36]	M	R184X	[b]	Yes/yes	Yes	No	No	—	Considered		—	—
P1 [37]	F	R184X, R200X	[b]	No/yes	Yes	No	No	—	Yes		—	—
P2 [37]	M	G43S	36/72m	Yes/no	No	Yes	Yes	HLH-94	Yes	Strabismus, hemiplegia, impaired speech	No	Yes
[38]	F	S18WfsX15	8/11y	Yes/yes	Yes	Yes	Yes	Other	No	Facial palsy, hemiplegia	—	Yes
1 [39]	M	R50KfsX35	—/5m	Yes/—	—	Yes	Yes	Modified HLH-94	No	Seizures	—	Yes
2 [39]	M	R50KfsX35	—/9m	Yes/—	—	Yes	Yes	Other	No	Spacticy, strabismus, seizure, hyperactive reflexes	—	Yes
3 [39]	M	R50KfsX35	—/4m	Yes/—	—	Yes	Yes	HLH-94	No	Seizures	—	Yes
4 [39]	F	R50KfsX35	—/3m	Yes/—	—	Yes	Yes	Other	No	Hypoactivity	—	Yes
5 [39]	F	R50KfsX35	—/2m	Yes/—	—	Yes	Yes	HLH-94	Yes	Hypotonicity, seizures	—	Yes
6 [39]	F	W73G	—/48m	Yes/—	—	Yes	Yes	HLH-94	Planned	Ataxia, strabismus	—	No
7 [39]	F	W73G	—/48m	Yes/—	—	Yes	Yes	HLH-94	Yes	Ataxia, strabismus, nystagmus	—	Yes
8 [39]	F	Q116X	—/4y	Yes/—	—	Yes	Yes	HLH-94	No	Nystagmus, coma, seizure	—	Yes
[41]	F	Q118X	4m/—	Yes/no	No	No	No	Other	Planned		—	—
1 [42]	M	Q118X splice error	5m/—	No/no	No	Yes	Yes	ATG	No	Repeated convulsive episodes, bilateral hemipareses, motor coordination and speech impaired. CT pathological	—	—
[43]	M	K134E	69m/—	Yes/yes	No	Yes	Yes	Other	No	Seizures, inability to walk, slurred speech, hypotonia	Yes	Yes
[44]	F	Q172NfsX2	3/3m	Yes/no	No	No	No	ATG, ITMTX	Yes		—	—
[45]	M	Q172NfsX2	5.5m/—	Yes/no	No	No	No	HLH-94	Yes		Yes	—
[46]	M	Q172NfsX2	3m/—	Yes/yes	Yes	No	No	HLH-94	No		No	—

[46]	M	Q172NfsX2	3m/—	Yes/yes	Yes	No	No	HLH-94	No		No	—
[47]	M	Q172NfsX2	3/3m	No/no	No	No	No	Dexa, VP16, CSA	Planned		—	—
[48]	F	R200X	32m/—	Yes/no	No	Yes	Yes	—	—	Drowsiness, inability to sit or stand, mild speech delay, cranial nerve palsy	No	Yes
P1 [49]	F	Del pro-ex5	—	Yes/yes	—	—	Yes	—	No	Delayed psychomotor development	—	—
P2 [49]	F	Del pro-ex5	—	Yes/yes	—	—	Yes	—	No	Delayed psychomotor development	—	—
P3 [49]	F	Del pro-ex5	—	Yes/yes	—	Yes	Yes	—	No	Delayed psychomotor development	—	—
P4 [49]	M	Del pro-ex5	1y/—	Yes/yes	—	—	Yes	—	No	Periodic episodes of seizures, mild cognitive delay	—	—
[50]	M		7m/—	Yes/no	No	—	Yes	ATG, ITMTX	No		No	—
Total				79%/60%	11/29, 38%	21/38, 55%	29/43, 67%		12/41, 29%			

Cons., reported parental consanguinity; Fam, reported familial disease; Neuro at diag, reported neurological alteration at diagnosis/start of treatment; Neuro during course, neurological alteration during the course of the disease; ITMTX, intrathecal methotrexate; ITPred, intrathecal prednisolone; ATG, anti-thymocyte globulin-based treatment; Dexa, dexamethasone; CSA, cyclosporin A; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; —, no data; m, months; y, years. [a]Patient described in the current study; [b]Had not developed any clinical disease at the time of the initial publication.

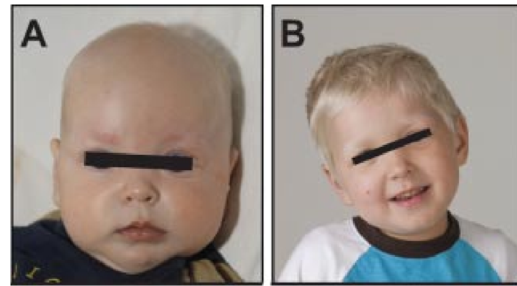

Fig. 3. The diagnosis of GS2 can easily be overlooked, particularly in fair-haired populations. The patient (B:1) is of Danish origin and has always had completely white hair. However, GS2 was not considered at 3 months of age when he developed a haemophagocytic syndrome (**A**). It was not until the time of HSCT at 15 months of age that the diagnosis of GS2 was suspected and subsequently confirmed by microscopic examination of the hair. **B**: The patient at the age of 4 years.

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