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Immunologic and clinical features of 25 Amish patients with *RMRP* 70 A→G cartilage hair hypoplasia ☆

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Abstract Cartilage-hair hypoplasia is a short limbed skeletal dysplasia associated with impairments in host-defense. To better understand the clinical heterogeneity of this disorder, we studied 25 Amish patients with homozygous mutations in *RMRP* (*RMRP* 70 A>G). Despite mutation homogeneity, eight (32%) patients had severe or recurrent infections, two (8%) of these children underwent bone-marrow transplantation for combined immunodeficiency, and the remainder were healthy. Features distinguishing patients who underwent bone marrow transplantation from others were shorter birth length, and lower serum IgG, undetectable serum IgA, and elevated circulating NK cells before 2 years of age. Irrespective of clinical phenotype, most patients had lymphopenia and reduced lymphocyte proliferation to mitogens *in vitro*. Our cohort analysis suggests that many patients with cartilage-hair hypoplasia are at risk for infection susceptibility particularly during the first 2 years of life. Gauging this risk is difficult, and thus careful monitoring of all patients with cartilage-hair hypoplasia is warranted.

Introduction

Cartilage-hair hypoplasia (CHH) is an autosomal recessive metaphyseal dysplasia caused by mutations in *RMRP* which encodes an untranslated RNA component of a mitochondrial ribonucleoprotein [1]. CHH is associated with a broad spectrum of immune dysfunction including severe combined immunodeficiency (SCID), hypogammaglobulinemia, chronic neutropenia, lymphopenia and a predisposition to lymphoma [2–5]. Previous studies describe CHH patients who suffered from recurrent bacterial infections or fatal complications of varicella [6–8].

Susceptibility to infection in CHH results from abnormal cellular and humoral immune responses [4–6,9–13]. Common laboratory findings are lymphopenia, a low CD4/CD8 ratio and reduced lymphocyte and fibroblast proliferation *in-vitro* [6,12,14,15]. Impaired antibody production has also been observed [5,9,13,16]. However, in a given individual, no combination of laboratory tests reliably predicts susceptibility to infection, autoimmunity or malignancy.

To better understand the immunological phenotype of CHH, we studied clinical and laboratory data from a cohort of

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25 Amish CHH patients homozygous for *RMRP* 70 A>G mutations. Our principal goal was to identify auxological or laboratory indices associated with clinically significant immune dysfunction. Our results provide a framework for preventative management of this vulnerable patient group.

Patients and methods

Patients

This study was approved by the Institutional Review Board of the Lancaster General Hospital. Written consent was obtained for molecular testing. The cohort consisted of 25 Old-Order Amish patients with CHH resulting from homozygous 70 A>G mutations in *RMRP* (mean age 7 years, range 9 months–21 years; 15 patients were female). All received primary care at the Clinic for Special Children, Strasburg, Pennsylvania. Patients were classified based on the following clinical phenotypes: autoimmune disease ($n=1$), combined immunodeficiency (CID; $n=2$), recurrent infections ($n=5$), and healthy with typical features of CHH ($n=17$). The patient with autoimmune disease suffered from juvenile rheumatoid arthritis (JRA); and both CID patients had bone marrow transplants (BMT). Importantly, both CID patients had cell mediated and humoral immune abnormalities with a severe clinical course; however, they differed from 'classical' SCID in that T-lymphocytes were detectable. Patients with recurrent infections had at least one of the following: a life threatening infection prior to age 2 years or more than two bacterial infections per year during the first 2 years of life. Healthy patients had neither severe, frequent, nor unusual infections.

Clinical methods

For 25 patients, we retrospectively reviewed medical history, growth data, infection history, and routine laboratory results. For ten patients, we prospectively obtained more detailed immune studies. Flow cytometry and *in vitro* stimulation of lymphocytes with phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (ConA) were performed at a reference laboratory (Mayo Medical Laboratories, Rochester, MN). Patient response to mitogens was compared to "control-of-the-day".

Molecular diagnostic methods

Total genomic DNA from whole blood was isolated using the PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. The *RMRP* target sequence was amplified by using specific oligonucleotide primers (Forward: 5′-AGG CCA CGC CCA CTC CCC GTA G-3′; Reverse: 5′-AGC CTG AGG TGA GGC ATC

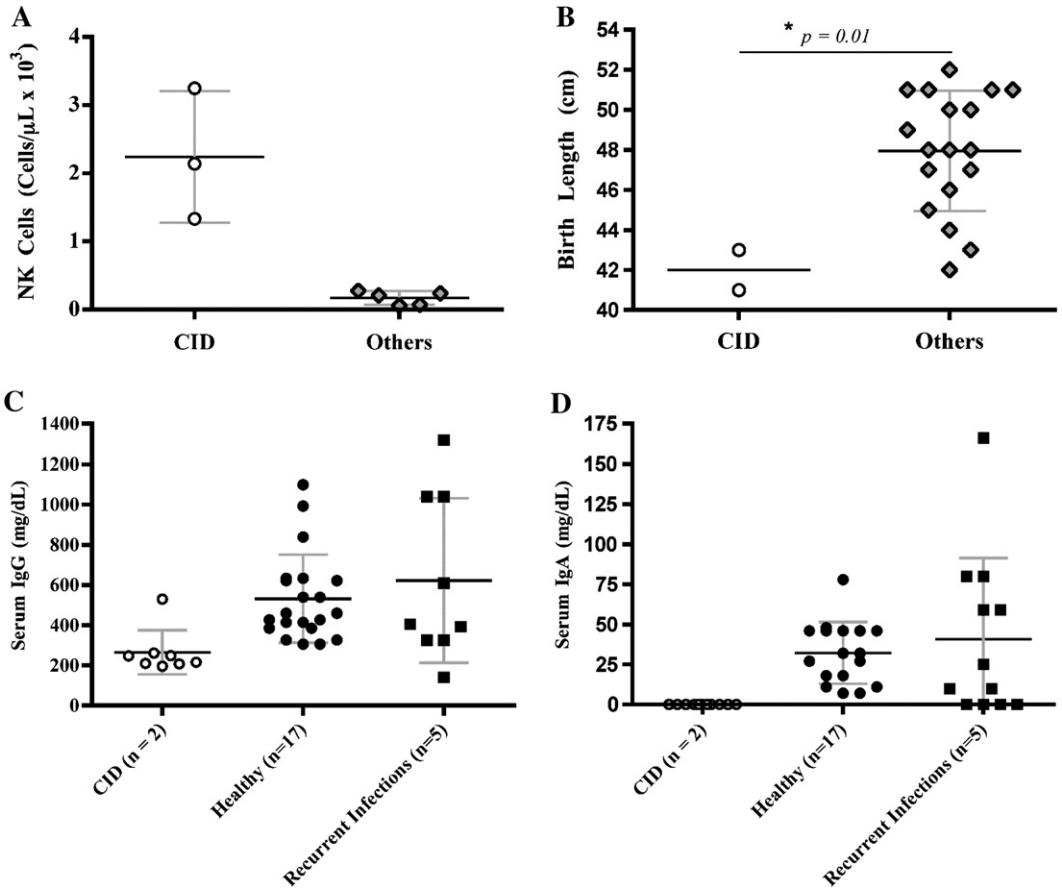

Figure 1 Features distinguishing CID patients from others within the cohort. Shown are means and standard deviations for: (A) serum NK cell ($CD16/56^{+}$) quantity within the first 2 years of life, (B) birth length, (C) serum IgG and (D) serum IgA within the first 2 years of life. ($^{*}p$-value by Students' t-test).

GCG T-3′) and 30–50 ng of genomic DNA from affected persons. PCR products were purified using QiaQuick columns (Qiagen, Valencia, CA, USA), as per manufacturer's instructions, and then sequenced using the BigDye Terminator cycle sequencing protocol (Applied Biosystems, Foster City, CA, USA). Extension products were then size-fractionated on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical methods

Statistical analyses were done with Prism 4 (GraphPad Software, San Diego, CA, USA) and data are shown as means ±standard deviations. Laboratory comparisons between the four phenotype groups included data from the CID patients prior to transplantation. Student's *t*-tests were performed to compare birth length groups (Fig. 1). Kaplan-Meier curves were constructed on the Amish cohort.

Results

Case summaries

Three vignettes depict the spectrum of clinical presentations within the cohort.

Case 1: combined immunodeficiency
An Amish boy was born at term with classical features of CHH, including short limbs and sparse hair. He presented with bacterial pneumonia at 4 months of age. Laboratory studies showed low levels of immunoglobulin G (IgG, 210 mg/dL), M (IgM, 16 mg/dL), and A (IgA, undetectable), and lymphopenia (absolute lymphocyte count (ALC) 350 cells/μL). He suffered recurrent otitis media, chronic diarrhea, and poor somatic growth. At 5 months of age, he had low levels of $CD4^+$ cells (1590 cells/μL; *normal 1800–4000*), $CD8^+$ cells (120 cells/μL; *normal 590–1600*), and an elevated natural killer cell count (NK cell, $CD16/56^+$, 3250 cells/μL; *normal 170–830*). At 14 months of age IgG (530 mg/dL) and IgM (69 mg/dL), were improved, but he had low counts of $CD4^+$ cells (1258 cells/μL; *normal 1300–3400*), and $CD8^+$ cells (115 cells/μL; *normal 620–2000*), with IgA deficiency. Unfractionated

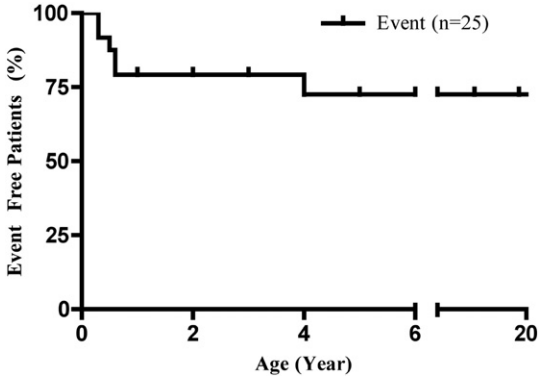

Figure 2 Morbidity and mortality among CHH patients. Time to event analysis for the Amish cohort, where events are defined as first hospitalization.

Table 1 Clinical features and infection history of the Amish CHH cohort

Patients with infectious Complications complications (*n*=8)			Other medical conditions found within the Cohort cohort (*n*=25)	
Patient category	Infection	Number affected	Diagnosis	Number affected
Autoimmune (*n*=1)	Disseminated parvovirus	1	Musculoskeletal	
	Recurrent otitis media	1	Short-limbed short stature	25
CID (*n*=2)	Thrush	2	Polyarticular JRA	1
	Recurrent otitis media	2	Scoliosis	1
	Bacteremia	1	Hematologic	
	Disseminated HSV	1	Nomocytic anemia	13
	Bacterial pneumonia	1	ITP	2
	H.flu. meningitis	1	AIHA	1
	CMV pneumonitis	1	Neutropenia (chronic)	1
Recurrent infections (*n*=5)	Thrush	3	Pulmonary	
	Recurrent otitis media	3	Asthma	4
	Sepsis	2	Gastroenteral	
	CMV pneumonitis	1	Hirschprung disease	3
	Interstitial pneumonia	1	Symptomatic GE reflux	2
	Recurrent sinusitis	1	Cardiac	
			Patent foramen ovale	2
			Atrial arrhythmia	1
			Endocrine	
			GH deficiency	2
			Neurologic	
			Cerebellar ataxia	1

(ITP)Immune thrombocytopenic purpura; (AIHA)autoimmune hemolytic anemia; (GH)growth hormone; (JRA)juvenile rheumatoid arthritis.

mitogen studies were markedly abnormal (ConA 11%; PHA 13%; PWM 7% of control values). After pre-conditioning with busulfan and cyclophosphamide, the child underwent BMT with an unrelated donor graft at 28 months. Human leukocyte antigen (HLA) and ABO antigens were matched, but Rh-D antigen was mismatched. Following transplantation, his course was complicated by intractable autoimmune hemolytic anemia (AIHA) from which he died at 39 months of age.

Case 2: clinically well with abnormal labs

An Amish boy with CHH presented at 3 months of age for routine well-child care. Laboratory studies revealed lymphopenia (1300 cells/μL). At 7 months of age his lymphocyte count remained low (1070 cells/μL) and he had severe neutropenia (8 cells/μL), hypogammaglobulinemia (IgG—268 mg/dL), abnormal mitogen responses (ConA—28% of control; PHA—27% of control; PWM—28% of control), and low levels of CD4 (231 cells/μL; *normal 1400—4300*), CD8 (23 cells/μL; *normal 500—1700*), and CD19 cells (176 cells/μL; *normal 610—2600*) with normal CD16/56 quantitation (276 cells/μL; *normal 160—950*). He was placed on immunoglobulin replacement with resolution of both his hypogammaglobulinemia (920 mg/dL) and neutropenia (3530 cells/μL). He is now 16 months old, and healthy. He has not required antimicrobial therapy.

Table 2 Lymphocyte quantitation for the Amish CHH cohort

Parameter	Parameter units	Normal range[24,25]	Patients (*n*)	CHH cohort
ALC	Cells × 10^3/mL			
Birth—6 months		3.4–9	11	2.3 ± 2.2
6—12 months		3.4–9	5	1.5 ± 0.55
1—2 years		3.6–8.9	7	1.9 ± 1.5
2—6 years		2.3–5.4	9	2.1 ± 0.9
6—12 years		1.9–3.7	3	1.7 ± 0.4
12—18+ years		1.4–3.3	4	1.7 ± 0.4
CD3	Cells × 10^3/mL			
Birth—6 months		2.4–5.6	0	NA
6—12 months		2.5–5.9	2	0.049—0.34
1—2 years		2.1–6.2	1	0.049—2.3
2—6 years		1.4–3.7	4	1.6 ± 0.68
6—12 years		1.2–2.6	1	0.85
12—18+ years		1–2.3	2	1.0—1.3
CD4	Cells × 10^3/mL			
Birth—6 months		1.6–4	2	0.25—1.6
6—12 months		1.4–4.3	2	0.034—0.23
1—2 years		1.3–3.4	1	0.023—1.1
2—6 years		0.7–2.2	4	0.63 ± 0.22
6—12 years		0.65–1.5	1	0.51
12—18+ years		0.53–1.3	2	0.5—0.51
CD8	Cells × 10^3/mL			
Birth—6 months		0.56–1.6	2	0.12—0.27
6—12 months		0.56–1.7	2	0.005—0.023
1—2 years		0.62–2	1	0.023—1.0
2—6 years		0.49–1.3	4	0.79 ± 0.40
6—12 years		0.37–1.1	1	0.22
12—18+ years		0.33–0.92	2	0.49—0.85
CD16/56	Cells × 10^3/mL			
Birth—6 months		0.17–0.83	2	2.1—3.3
6—12 months		0.16–0.95	2	0.058—0.28
1—2 years		0.18–0.92	1	0.071—0.28
2—6 years		0.13–0.72	4	0.33 ± 0.23
6—12 years		0.10–0.48	1	0.42
12—18+ years		0.07–0.48	2	0.35—0.5
CD19	Cells × 10^3/mL			
Birth—6 months		0.3–3	0	NA
6—12 months		0.61–2.6	2	0.023—0.18
1—2 years		0.72–2.6	1	0.18—0.75
2—6 years		0.39–1.4	4	0.31 ± 0.27
6—12 years		0.27–0.86	1	0.056
12—18+ years		0.11–0.57	2	0.04—0.079

Values are reported as mean ± standard deviation or as a range when $n < 3$.
CD3—total T-cells; CD4—helper T-cells; CD8—cytotoxic T-cells; CD16/56—NK cells; CD19—B-cells; ALC—absolute lymphocyte count.

Case 3: autoimmune disease
An Amish girl with classical physical stigmata of CHH presented at 7 months of life with a diffuse, erythematous rash accompanied by prolonged fever, hepatosplenomegaly, and large joint arthritis. Laboratory studies showed an erythrocyte sedimentation rate (ESR) >100 mm/h, a leukocyte count of 55,000 cells/μL, a ferritin level of 25,000 mg/dL and normocytic anemia. Acute serology for parvovirus B19 was positive, and a bone marrow biopsy revealed erythroid hypoplasia without evidence of malignancy. Treatment with prednisone initially led to resolution of her anemia, constitutional symptoms, and arthritis. However, she had a chronic remitting course, and at 12 years of age, her JRA is controlled with anti-tumor necrosis factor therapy. Recent immunological assessment revealed normal immunoglobulin studies (IgG—1818 mg/dL; IgA—165 mg/dL; IgM—84 mg/dL), normal NK cell counts (423 cells/μL; *normal 100—480*), lymphopenia (ALC—1360 cells/μL; *normal 1900—3700*), and low T-lymphocyte counts (CD4—513 cells/μL; *normal 650—1500*; CD8—222 cells/mL; *normal 370—1100*). She has two siblings with CHH who do not have autoimmune disease. One sibling had recurrent sinus infections as a young child but is now healthy. The other has remained well without infections or autoimmunity.

Host defense in CHH

Infections were common, affecting 8 of 25 patients (32%), and ranged in severity from recurrent otitis media to bacterial meningitis (Table 1). Six patients (24%) had life threatening infections (*H. influenzae* meningitis, sepsis, bacterial pneumonia, interstitial pneumonia, cytomegalovirus pneumonitis, and parvovirus B19 infection with aplastic crisis). Among 8 patients, there were a total of 13 hospitalizations for management of infections during the first 4 years of life (0.5 hospitalizations/patient/year; earliest hospitalization at 7 weeks). Two patients required BMT, and three received immunoglobulin replacement (2 CID, and 1 hypogammaglobulinemia).

Two children presented with CID. One died from AIHA (Case 1). The second child also had low immunoglobulin levels, lymphocyte subpopulations and poor lymphocyte proliferation to mitogens early in life. She was hospitalized at 3.5 months with pneumonia, at 7 months with respiratory distress and febrile neutropenia (ANC = 78 cells/μL), and at 8.5 months with *H. influenzae* meningitis despite receiving 3 doses of *H. influenzae type b* conjugate vaccine. She underwent matched unrelated BMT following busulfan and cyclophosphamide conditioning at the age of 14 months. At 6 years of age she is healthy with normal lymphocyte quantity and function; however, she does have chronic idiopathic thrombocytopenic purpura.

Serious events

Figure 2 depicts the time to first hospitalization ($n = 7$) for Amish CHH patients. Most initial serious events occurred by age 1 year. Like previously reported cases, the majority (58%) of all serious events in our cohort occurred before age 2 years [4,7,8,16–23]. Some of our patients also suffered death ($n = 1$) and multiple hospitalizations ($n = 3$) early in life.

Laboratory studies

The majority of CHH patients had low age-adjusted absolute lymphocyte counts (Table 2 and Fig. 3) [24,25]. Absolute lymphocyte counts did not clearly distinguish phenotype groups, but they tended to be lower in the CID and recurrent infection groups (Fig. 3). Within the first 2 years of life, natural killer cell ($CD16/56^+$) count was elevated in the CID

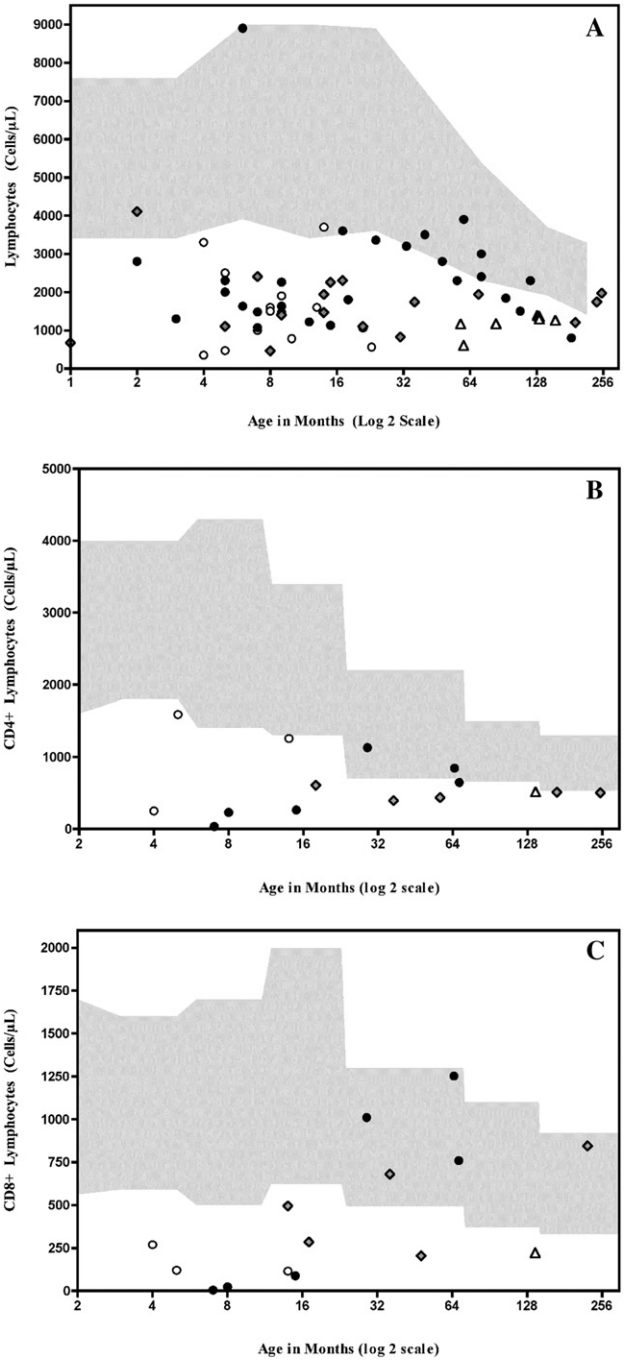

Figure 3 Spectrum of selected hematologic indices for age and clinical phenotype. (A) Absolute lymphocyte counts, (B) $CD4^+$ lymphocytes, and (C) $CD8^+$ lymphocytes are shown. Phenotype groups: ● Healthy; ♦ recurrent infections; ○ CID; ▲ autoimmune.

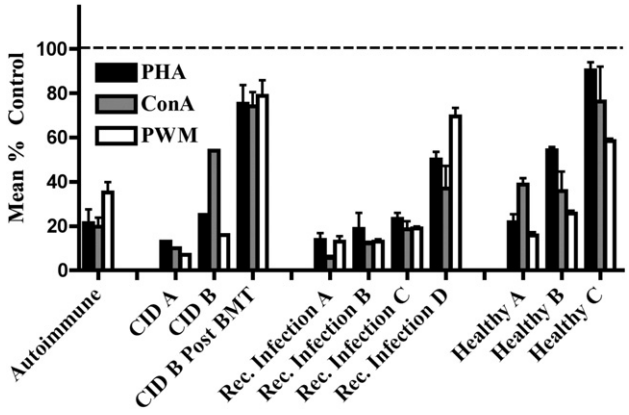

Figure 4 Mitogen induced lymphocyte in 10 CHH patients compared to controls (line at 100% = control). Values for CID patient B are shown before and after transplantation. Values for CID patient A are shown before transplantation only. (PHA—phytohaemmaglutinin A; ConA—concanavalin A;PWH—pokeweed mitogen).

patients compared to others within the cohort (Fig. 1). Other lymphocyte subpopulations and hematologic parameters for CHH patients were abnormal for age (Table 2, Fig. 3), but did not differ among phenotype groups (data not shown) [24,25]. Mitogen-induced proliferation of unfractionated lymphocytes was poor for most CHH patients, and proliferative responses did not clearly correlate with clinical phenotype (Fig. 4).

Low serum IgG, and undetectable serum IgA were distinguishing features of CID patients within the first 2 years of life (Fig. 1) [26]. In all other phenotype groups IgA and IgM production developed normally; whereas, IgG production was variable (Table 3, Fig. 5).

In one CID patient, protective *H. influenzae* titers failed to develop after three vaccine doses and *H. influenzae* meningitis. However, overall we did not find a correlation between specific antibody production and clinical phenotype. Mean titers to the pneumococcal 7-valent conjugate vaccine (Wyeth) were equal to or greater than reported normal values for serotypes 4, 18C and 19F, whereas, response to strains 6B, 14, and 23F were low (Fig. 6) [27,28]. Titers to *C. diphtheria* and *C. tetani* were generally at or above protective levels (Fig. 6), and responses to *H. influenzae* were variable (Fig. 6) [29–33].

Growth

Birth lengths were available for 15 of the healthy patients, 3 of the patients in the recurrent infection group, and both CID patients. The CID patients were significantly shorter at birth compared to both healthy individuals and those with recurrent infections (Fig. 1B).

Discussion

CHH is classified as a disorder of bone and ectoderm. However, morbidity and mortality is most directly a consequence of immune dysfunction. Infections in children and hematologic malignancies in adults are particularly important problems [7]. In a large Finnish cohort of CHH patients, 60 (56%) had frequent or severe infections, and six of these patients (5.6%) died of infections [19]. In comparison, eight of our patients (32%) were unusually susceptible to infection, in two cases warranting

Table 3 Quantitative immunoglobulins for the Amish CHH cohort

Parameter	Parameter units	Normal range[26]	Patients (*n*)	CHH cohort (*n* = 25)
Serum IgG	mg/dL			
Birth–6 months		172–906	9	397 ± 173
6–12 months		215–1069	7	385 ± 262
1–2 years		345–1051	6	496 ± 312
2–6 years		424–1280	4	897 ± 323
6–12 years		608–1572	1	1818–2030
12–18+ years		639–1349	2	1320–1770
Serum IgA	mg/dL			
Birth–6 months		1.3–73	9	14 ± 19
6–12 months		8.1–90	4	30 ± 32
1–2 years		14–123	5	30 ± 39
2–6 years		14–202	3	81 ± 44
6–12 years		33–236	1	112–165
12–18+ years		70–312	2	161–203
Serum IgM	mg/dL			
Birth–6 months		20–102	9	52 ± 46
6–12 months		35–149	4	113 ± 115
1–2 years		43–173	5	81 ± 75
2–6 years		45–196	3	106 ± 7
6–12 years		48–242	1	79–84
12–18+ years		56–352	2	82–101

Values are reported as mean ± standard deviation or as a range when $n < 3$.

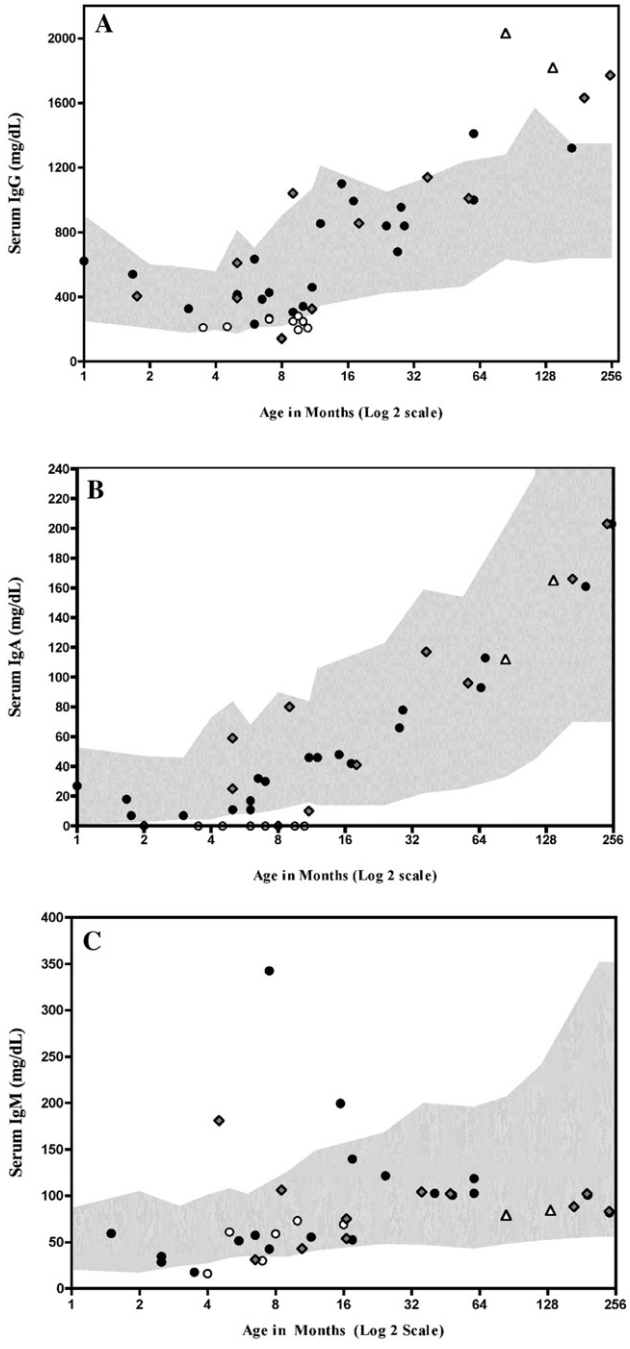

Figure 5 Spectrum of serum immunoglobulin production for age and clinical phenotype. (A) Serum IgG. (B) IgA, and (C) IgM are shown. Phenotype groups: ● Healthy; ♦ recurrent infections; ○ CID; ▲ autoimmune.

treatment with BMT. The remaining six were hospitalized early in life with no subsequent problems. None of our patients have had a malignancy, but this is a young cohort (9 mos.–21 yrs.). Given the heterogeneity of immune function, we tried to determine if *all* CHH patients are immunodeficient during a critical window of development; or, if certain affected individuals are unusually susceptible to infection.

Despite numerous reports of immunodeficiency in CHH, the molecular basis for dysfunction remains poorly understood. The yeast *RMRP* ortholog *nme1* participates in ribosomal assembly and cell cycle regulation; however, the *RMRP* gene product's role in immune function is not known [34]. Recent studies show that the position of an *RMRP* mutation may affect susceptibility to infection, but our cohort displays clinical heterogeneity despite mutation homogeneity and similar environmental exposures [35–38]. In contrast, individuals with homozygous mutations in *RMRP* display striking diversity in their clinical phenotype even within a family. This suggests that the type and position of *RMRP* mutations alone are not sufficient to explain the susceptibility to infection and clinical heterogeneity seen in CHH patients.

Assessing host defense in CHH is difficult, because hematologic indices, responses to mitogens, and markers of humoral immunity do not consistently correlate with disease risk (Figs. 3–5). Similar to other reports of CHH, many individuals from this cohort had normal kinetics of immunoglobulin production, and intact specific antibody formation after vaccination (Figs. 5 and 6) [5,22,39,40]. This was true in each phenotype group, including patients with CID, and suggests that *RMRP* 70 A>G lymphocytes can proliferate and form memory responses to both thymus-dependent and thymus-independent antigens *in vivo*, despite abnormal *in vitro* proliferation. However, quantitative immunoglobulin production was abnormal in some individuals and IgA deficiency in CID patients was striking. Our observations and those of others suggest that sustained absence of serum IgA may be a risk factor for severe immune deficiency in CHH [20,41,42].

It is troubling that individuals with CHH who have CID were not easily distinguished from those who were healthy based upon laboratory investigations. Although our CHH patients with CID had severe infections early in life, their lymphocyte counts, proliferation studies, and serum immunoglobulin levels overlapped considerably with healthy children from the cohort (Figs. 3–5) [17,20,41,42]. We did find that our CID patients differed with respect to birth length and quantity of serum IgG, IgA and NK cells within the first 2 years of life (Fig. 1). Although our data were not in sufficient quantity to determine differences between phenotype groups for all parameters, they may suggest the importance of following cellular as well as humoral immune parameters when determining risk for an individual with CHH. At present, we cannot clearly identify markers to justify presymptomatic BMT, which ideally should be done within the first few months of life to optimize engraftment and survival [43,44].

It is unfortunate that we do not have information about pretransplantation B cell quantity in our CID patients. In general, CHH patients from our cohort had normal immunoglobulin levels throughout childhood and evidence of response to vaccination (Table 3, Figs. 5 and 6); however, patients with CID had abnormally low IgG and IgA levels within the first 2 years of life as described above (Fig. 1). To address the issue of humoral immunity in CHH, future investigations should include measures of specific B cell indices to determine whether these are helpful markers of infection risk.

Previous reports of CHH indicate a marked susceptibility to varicella, with severe or fatal reactions [4,8,22]. These reports and the marked elevation of NK cells in our CID

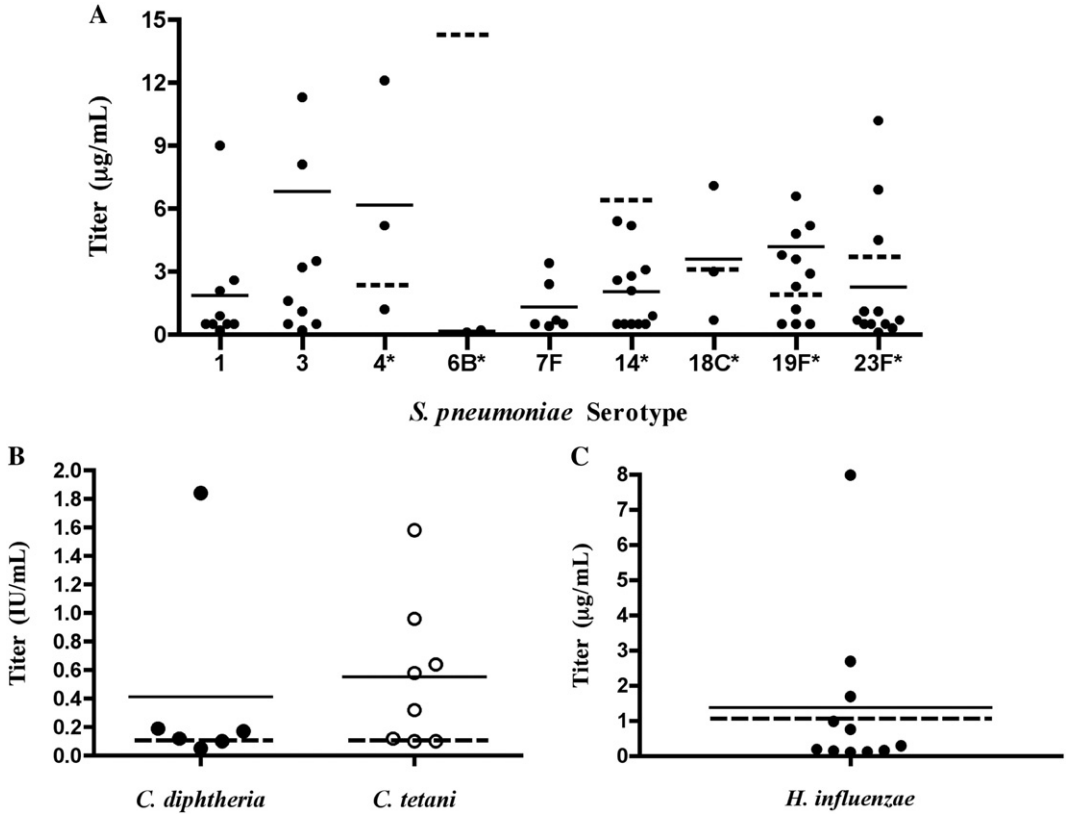

Figure 6 Titers following vaccination to (A) *S. pneumoniae* (B) *C. diphtheria*, *C. tetani* and (C) *H. influenzae*. Solid horizontal bars represent the geometric mean values for the cohort. Dashes bars represent: literature reported means for healthy children (*S. pneumoniae* graph), or levels considered to be protective (*C. diphtheria*, *C. tetani* and *H. Influenzae* graphs) Asterisks following *S. pneumoniae* serotype indicate inclusion in the pneumococcal 7-valent conjugate vaccine. (Literature reported means: *S. pneumoniae* in mg/mL: 4=2.38; 6B=14.45; 14=6.52; 18C=3.43; 19F=2.07; 23F=3.82 [27,28], Protective levels: *C. diphtheria*, *C. tetani*=0.1 IU/mL [29,30] and *H. influenzae*=1 mg/mL. [31–33]).

patients (Fig. 1A) may suggest a defect in cytotoxicity. However, previous studies of *in vitro* NK cell function in CHH patients with a clinical history of severe varicella infection showed normal killing of target cells [45,46]. Several patients within our cohort developed varicella, but none required hospitalization or antiviral therapy to achieve clinical resolution. In 13 patients studied, 7 (54%) had protective titers to varicella; only one patient was immunized. These data suggest that some CHH patients may safely receive vaccination for varicella. The significance and mechanism of the elevated NK cell counts in the CID patients is unknown; but, this phenomenon occurs in other primary immunodeficiency diseases [47,48].

Previous reports indicate that birth length correlates with subsequent infection risk, whereas height at ages 2–6 years and final adult height do not [19]. Our observations are consistent with this. The two CID patients had significantly lower birth lengths compared to the other clinical phenotypes (Fig. 1), but postnatal growth was similar among all clinical phenotypes. These findings indicate that prenatal growth and immune function before age 2 may be linked in CHH.

Although many individuals with CHH survive into adulthood without incident, there appears to be a period of vulnerability to infection within the first 2 years of life, with bacterial pneumonia as the leading cause of death [4,7,8,16–23]. In six of seven (86%) patients who were hospitalized (Fig. 2) infections prompting the first hospitalization occurred before 9 months of age. These data suggest that immunodeficiency in CHH may be dynamic, changing in an individual over time, and that all affected infants should be monitored carefully for signs of infection susceptibility during the first 2 years of life.

In summary, CHH is a diverse syndrome with unique immunological features. We have identified differences among phenotype groups within this cohort (e.g. early life IgA, IgG, NK cell quantity and birth length) which may allow for risk stratification. Patients with CHH should receive careful routine follow up and early, aggressive treatment of infections with particular vigilance during the first 2 years of life (Fig. 7).

Future work on managing patients with CHH should focus upon understanding the maturation and diversity of immune function with a focus on presymptomatic diagnosis of severe immunodeficiency. Identification of children who can safely receive live vaccines, especially varicella vaccine, will also be important. Further understanding of the clinical heterogeneity in CHH will likely come from identifying other genes that modify immune defense, by tracing the development of lymphocyte repertoires early in

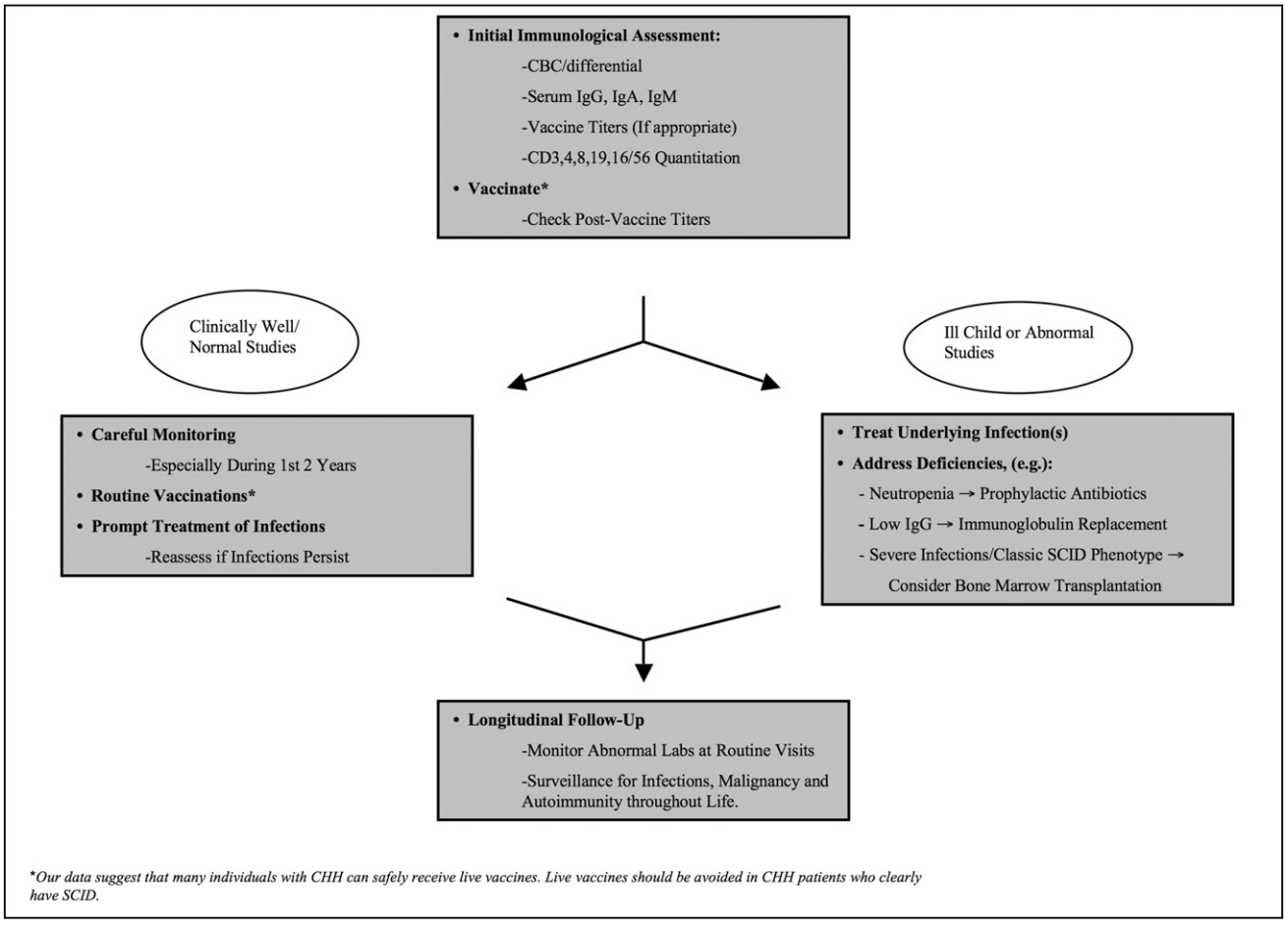

Figure 7 Suggested paradigm for initial and long-term management of individuals with Cartilage-hair hypoplasia.

life, and by more fully understanding the function of the *RMRP* gene product.

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