Doctoral Programme in Clinical Research,

Children's Hospital,

University of Helsinki and Helsinki University Hospital,

and Folkhälsan Institute of Genetics

Helsinki, Finland

IMMUNODEFICIENCY IN CARTILAGE-HAIR HYPOPLASIA: CORRELATION WITH PULMONARY DISEASE, INFECTIONS AND MALIGNANCY

Svetlana Vakkilainen

Academic dissertation

To be publicly discussed,

with the permission of the Faculty of Medicine

of the University of Helsinki, in the Hattivatti lecture hall,

New Children's Hospital on the 19th of June 2019, at 12:00 noon

Helsinki 2019

Supervisors

Professor Outi Mäkitie
Children's Hospital, University of Helsinki and Helsinki University Hospital
Helsinki, Finland

Docent Paula Klemetti
Children's Hospital, University of Helsinki and Helsinki University Hospital
Helsinki, Finland

Reviewers

Docent Leena Kainulainen
Department of Pediatrics and Adolescent Medicine
University of Turku and Turku University Hospital
Turku, Finland

Docent Johanna Lempainen
University of Turku and Turku University Hospital
Turku, Finland

Opponent

Professor Andrew Cant
University of Newcastle
Newcastle, United Kingdom

ISBN 978-951-51-5227-5 (paperback)
ISBN 978-951-51-5228-2 (PDF)
http://ethesis.helsinki.fi

Unigrafia
Helsinki 2019

The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

“Science is continually correcting what it has said.”

Victor Hugo

Table of Contents

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I **Kostjukovits S**, Degerman S, Pekkinen M, Klemetti P, Landfors M, Roos G, Taskinen M, Mäkitie O.
Decreased telomere length in children with cartilage-hair hypoplasia.
J Med Genet, 54, 365-370 (2017).

II **Kostjukovits S**, Klemetti P, Valta H, Martelius T, Notarangelo LD, Seppänen M, Taskinen M, Mäkitie O.
Analysis of clinical and immunologic phenotype in a large cohort of children and adults with cartilage-hair hypoplasia.
J Allergy Clin Immunol, 140, 612-614.e5 (2017).

III **Kostjukovits S**, Klemetti P, Föhr A, Kajosaari M, Valta H, Taskinen M, Toiviainen-Salo S, Mäkitie O.
High prevalence of bronchiectasis in patients with cartilage-hair hypoplasia.
J Allergy Clin Immunol, 139, 375-378 (2017).

IV **Vakkilainen S**, Taskinen M, Klemetti P, Pukkala E, Mäkitie O.
A 30-year prospective follow-up study reveals risk factors for malignancies and early death in cartilage-hair hypoplasia
Submitted in December 2018.

The publications are referred to by their Roman numerals in the text. The publications have been reprinted with the permission of their copyright holders.

ABBREVIATIONS

CHH cartilage-hair hypoplasia

CID combined immunodeficiency

HRCT high-resolution computed tomography

HSCT hematopoietic stem cell transplantation

Ig immunoglobulin

IGRT immunoglobulin replacement therapy

MRI magnetic resonance imaging

PID primary immunodeficiency disorders

rho Spearman's rank correlation coefficient

RMRP RNA component of the mitochondrial RNA-processing endoribonuclease

RTI respiratory tract infections

RTL relative telomere length

SCID severe combined immunodeficiency

SD standard deviation

SIR standardized incidence ratio

SMR standardized mortality ratio

ABSTRACT

Background. Cartilage-hair hypoplasia (CHH) is a rare chondrodysplasia with short stature, hair hypoplasia, combined immunodeficiency and increased risk of malignancy. The non-coding RNA gene *RMRP* is mutated in CHH, which leads to several cellular derangements, including cell cycle impairment. However, the spectrum and evolution of the clinical manifestations and the pathogenesis of CHH are incompletely understood. The degree of immunodeficiency is highly variable, and previous studies have failed to establish clear clinical or laboratory correlates of disease severity. Patients with CHH display increased mortality due to infections and malignancies, however, factors associated with mortality remain unrecognized. Respiratory infections can induce the development of bronchiectasis, but lung changes have not been systematically studied in CHH.

Objectives. We performed detailed immunologic evaluation of a large cohort of patients with CHH, explored the prevalence of bronchiectasis and compared diagnostic lung imaging modalities, and conducted a prospective long-term follow-up study to identify factors associated with adverse outcomes. We also investigated the role of telomere machinery in the pathogenesis of CHH.

Subjects and methods. We repeatedly recruited Finnish CHH patients, identified through the Finnish Chondrodysplasia Registry (n = 104-110), and included all subjects who consented to participate (n = 56-80). Patients were interviewed and clinically examined, and blood samples were collected. Additional data were obtained from hospital records, the Finnish National Health Databases, the Cancer Registry and the Cause-of-death Registry of Statistics Finland. We evaluated patients' blood samples for a range of immunologic parameters (n = 56), and for relative telomere length (RTL) by quantitative-PCR method (n = 48). Blood samples from the first-degree relatives and DNA samples from healthy controls were also used for RTL measurements. A subgroup of patients (n = 34) underwent lung imaging by high-resolution computed tomography (HRCT) and magnetic resonance (MRI).

Results. Common immunological findings in 56 patients with CHH included: 1) decreased thymic naive, naive CD4+ and CD8+ T cells; 2) increased activated CD4+, central memory CD4+ and effector memory CD8+ T cells; 3) normal regulatory T cells; 4) decreased naive, transitional and memory B cells; 5) increased activated B cells. Specific antibody deficiency was demonstrated in the majority of patients immunized with unconjugated 23-valent pneumococcal vaccine (Pneumovax®). No significant correlations were observed between clinical and laboratory features. Patients with CHH demonstrated significantly shorter median RTL compared with healthy controls. RTL correlated with age in carriers and non-carriers of *RMRP* mutations, but not in patients, due to the shorter telomeres in children with CHH. HRCT showed bronchiectasis in 10/34 patients (29%) and MRI scores correlated significantly with HRCT scores. In the prospective study of 80 subjects with CHH, the median duration of follow-

up for the surviving patients was 29.2 years (range 25.6 – 31.0 years). In a significant proportion of patients (17/79, 22%), clinical features of immunodeficiency progressed over time, including six cases of adult-onset immunodeficiency. Of the 15 subjects with non-skin malignancy, eight demonstrated no preceding symptoms of immunodeficiency. Altogether 20/80 patients had died during the follow-up, and causes of death included pneumonia (n = 4), malignancy (n = 7) and pulmonary disease (n = 4). Increased mortality was associated with severe short stature at birth, Hirschsprung disease, pneumonia, autoimmunity and symptoms of combined immunodeficiency. In addition, warts in adulthood and actinic keratosis were associated with the development of skin cancer. Patients with shorter birth length developed malignancy at an earlier age.

Conclusions. Study patients demonstrated specific abnormalities in B and T cell compartments. Antibody responses to polysaccharide antigens were impaired in the majority of tested patients. Clinical features did not correlate with laboratory parameters. Patients with CHH showed high prevalence of bronchiectasis, and lung evaluation is indicated also in those without apparent immunodeficiency. Lung MRI was comparable to HRCT in the assessment of bronchiectasis and can be implemented in the follow-up of lung changes. Telomere length was decreased in subjects with CHH, especially children. Patients with CHH demonstrated high mortality due to infections and malignancies, but also from lung disease. Some subjects presented with adult-onset immunodeficiency or malignancy without preceding symptoms of disrupted immunity, warranting careful follow-up and screening for cancer even in asymptomatic patients. We provided clinicians with the risk factors for adverse outcomes to assist in management decisions and we suggested implication of our results for the management of Finnish patients with CHH.

TIIVISTELMÄ

Tausta. Rusto-hiushypoplasia (RHH) on harvinainen luustodysplasia, jonka oireisiin kuuluvat kasvuhäiriön lisäksi hiusten hentous, kombinoitu immuunipuutos ja lisääntynyt syöpäriski. RHH johtuu ei-koodaavan RNA-geenin *RMRP*-mutaatioista, joiden seurauksena solusykli häiriintyy, mutta taudin tarkempia mekanismeja ei vielä ymmärretä. Immuunipuutoksen aste on erittäin vaihteleva eikä aiemmissa tutkimuksissa ole pystytty osoittamaan kliinisten oireiden tai laboratorioarvojen yhteyttä taudin vaikeusasteeseen. RHH:aa sairastavilla potilailla on lisääntynyt kuolleisuus infektiotauteihin ja maligniteetteihin, mutta kuolleisuuteen vaikuttavia tekijöitä ei tunneta. RHH:aa sairastaville potilaille, joilla on hengitystieoireita, voi kehittyä bronkiektasioita, mutta keuhkojen muutoksia RHH:ssa ei ole tutkittu järjestelmällisesti.

Tutkimuksen tarkoitus. Suoritimme yksityiskohtaisia immunologisia tutkimuksia laajassa RHH:aa sairastavien potilaiden kohortissa. Tutkimme bronkiektasioiden esiintyvyyttä ja keuhkojen eri kuvantamismenetelmiä. RHH-potilaiden kuolleisuuteen ja syöpäsairastumiseen liittyvien tekijöiden tunnistamiseksi suoritimme potilaille 30 vuoden seurantatutkimuksen. Tutkimme myös telomeerien roolia RHH:n patogeneesissä.

Aineisto ja menetelmät. Suomen Luustodysplasiarekisterin kautta tunnistettiin toistuvasti Suomen RHH:aa sairastavat potilaat (n = 104–110), joista tutkimukseen mukaan otettiin kaikki suostumuksensa antaneet (n = 56–80). Potilaita haastateltiin ja tutkittiin kliinisesti ja heiltä otettiin verinäytteitä. Lisätietoja kerättiin potilaiden sairaskertomuksista, Hilmo- ja Avohilmo-järjestelmästä ja Syöpärekisteristä sekä Tilastokeskuksesta. Potilaiden verinäytteistä tutkittiin useita immunologisia parametreja (n = 56) sekä suhteellinen telomeerien pituus (STP) kvantitatiivisella PCR-menetelmällä (n = 48). STP mitattiin myös potilaiden ensimmäisen asteen sukulaisten verinäytteistä ja terveiden henkilöiden DNA-näytteistä. Osalla potilaista (n = 34) kuvattiin keuhkoja tietokonetomografialla (TT) ja magneettikuvauksella (MK).

Tulokset. Tyypilliset immunologiset löydökset 56 potilaan kohortissa olivat: 1) vähentyneet tyymus-naiviit, naiivit CD4+ ja CD8+ -T-solut, 2) lisääntyneet aktivoituneet CD4+, keskusmuisti-CD4+ ja efektorimuisti-CD8+ -T-solut, 3) normaalit regulatoriset T-solut, 4) vähentyneet naiivit, transitionaalit ja muisti-B-solut, 5) lisääntyneet aktivoidut B-solut. Spesifinen vasta-ainepuutos osoitettiin suurimmalla osalla potilaista, jotka olivat saaneet 23-valenttisen, ei-konjugoidun pneumokokkirokotteen (Pneumovax®). Oireiden ja laboratorioarvojen välillä ei havaittu merkittäviä korrelaatioita. Mediaani-STP oli potilailla huomattavasti lyhyempi kuin verrokeilla. STP korreloi iän kanssa *RMRP*-mutaatioiden kantajilla ja ei-kantajilla, mutta ei potilailla, johtuen lyhyemmistä telomeereista RHH:aa sairastavilla lapsilla. TT-kuvauksella todettiin bronkiektasioita 10/34 potilaalla (29 %), ja MK tulokset korreloivat merkittävästi TT-tulosten kanssa. 80 potilaan prospektiivisessa

tutkimuksessa mediaaniseurannan kesto oli 29,2 vuotta (vaihtelu 25,6–31,0 vuotta). Merkittävällä osalla potilaista (17/79, 22 %) immuunipuutos eteni ajan myötä; kuudelle heistä kehittyi myöhäinen immuunipuutos aikuisiässä. Yli puolella syöpään (ihosyöpä pois lukien) sairastuneista potilaista (8/15, 53 %) ei ollut edeltäviä immuunipuutoksen oireita. Seurannan aikana kuoli yhteensä 20/80 potilasta, mm. keuhkokuumeeseen (4), syöpään (7) ja keuhkosairauksiin (4). Kuolleisuuteen liittyi useita riskitekijöitä, mukaan lukien lyhyempi syntymäpituus, Hirschsprungin tauti, keuhkokuume, autoimmuunitaudit ja kombinoitu immuunipuutos. Lisäksi aikuisiän syylillä ja aurinkokeratoosilla oli yhteyttä ihosyövän kehittymiseen. Ne potilaat, joiden syntymäpituus oli lyhyempi, sairastuivat maligniteettiin nuoremmalla iällä.

Johtopäätökset. Potilailla osoitettiin tiettyjä poikkeavuuksia B- ja T-soluissa. Useimmilla potilailla vasta-ainevasteet polysakkaridiantigeeneja kohtaan olivat heikentyneitä. Oireiden ja laboratorioparametrien välillä ei havaittu merkittäviä yhteyksiä. Potilailta löytyi usein bronkiektasioita, ja keuhkojen arviointi on aiheellista myös niillä, joilla ei ole immuunipuutoksen merkkejä. Keuhkojen MK-tulokset olivat verrattavissa TT-tuloksiin bronkiektasioiden arvioinnissa, ja MK:ta voidaan käyttää keuhkomuutosten seurannassa. Telomeerit olivat lyhyempiä RHH:aa sairastavilla potilailla, erityisesti lapsilla. Potilailla oli korkea kuolleisuus infektioihin ja maligniteetteihin, mutta myös keuhkosairauksiin. Osalle potilaista kehittyi myöhäinen immuunipuutos tai syöpä ilman edeltäviä merkkejä häiriintyneestä immuniteetista, mikä on peruste tarkkaan seurantaan ja syövän seulontaan myös oireettomilla potilailla. Kuvasimme tutkimuksissa useita kuolleisuuden riskitekijöitä, jotka helpottavat hoitopäätösten tekemistä ja toimivat perustana suomalaisten RHH:aa sairastavien potilaiden hoitosuosituksille.

1. INTRODUCTION

Primary immunodeficiency disorders (PID) comprise a heterogenous group of defects in the immune system. Several of them, including cartilage-hair hypoplasia (CHH, MIM # 250250), present with skeletal dysplasia among other features.

After the first description by Maroteaux et al in 1963, McKusick et al reported CHH in a series of 77 subjects of Amish origin in 1965 (Maroteaux, et al. 1963, McKusick, et al. 1965). These studies highlighted the main clinical characteristics of CHH, such as the short-limbed short stature, sparse and fine hair, unusual susceptibility to infections and intestinal defects. This rare chondrodysplasia was soon recognized to be overrepresented in Finland (Perheentupa 1972) and further studies described over 100 Finnish patients in 1990s (Makitie 1992, Makitie and Kaitila 1993).

The genetic background for CHH was uncovered in 2001 by Ridanpää et al who showed that mutations in the *RMRP* gene underlie this autosomal recessive disease (Ridanpaa, et al. 2001). *RMRP* was the first described disease-associated non-coding RNA gene. RNA component of the mitochondrial RNA-processing endoribonuclease (RMRP) is involved in gene regulation, rRNA and mRNA processing, and abnormal RMRP function induces the impairment of cell proliferation and differentiation (Rogler, et al. 2014, Thiel, et al. 2005). However, the pathogenesis of CHH remains poorly characterized.

Currently, CHH is classified as a syndromic immunodeficiency, in which metaphyseal chondrodysplasia is associated with combined immunodeficiency (CID), anemia, hair hypoplasia, increased incidence of malignancies and Hirschsprung disease. The incidence of CHH is highest among the Amish (1-2 in 1,000 births) and Finnish (1 in 23,000 births) populations, outside which CHH is a rare entity, providing a unique opportunity to study this disease in Finland (Makitie 1992).

Immunodeficiency in CHH is highly variable and does not correlate with the type of mutations (Kavadas, et al. 2008, Makitie and Kaitila 1993). Except for association between increased susceptibility to infections with Hirschsprung disease and shorter birth length (Makitie and Kaitila 1993, Makitie, et al. 2001), no prognostic factors in CHH have been described. This complicates management decisions and the selection of patients who would benefit from early hematopoietic stem cell transplantation (HSCT).

We addressed the pathogenesis, immunologic phenotype, lung disease and malignancies, and factors associated with mortality in patients with CHH.

2. REVIEW OF THE LITERATURE

2.1. Primary immunodeficiency

PID comprise a heterogenous group of over 350 disease entities that are characterized by the intrinsic defects in the immune system (McCusker, et al. 2018, Ochs and Petroni 2018, Picard, et al. 2018). PID are currently categorized into eight broad categories according to the common phenotype: 1) CID, 2) CID with syndromic features, 3) predominantly antibody deficiencies, 4) diseases of immune dysregulation, 5) defects of phagocyte number or function, 6) defects in intrinsic and innate immunity, 7) autoinflammatory diseases, and 8) complement deficiencies (Picard, et al. 2018).

The increasing number and diversity of PID has shifted diagnostic algorithms towards the early implementation of genetic tools (Leiding and Forbes 2019). However, thorough clinical history and examination, and measurement of basic laboratory immunologic parameters, remain the cornerstone of initial PID diagnostics (Sanchez-Ramon, et al. 2019). Cellular immunity is first evaluated with blood counts of neutrophils, lymphocytes, eosinophils and monocytes, as well as flow cytometry labeling of lymphocyte subpopulations (CD3+, CD4+ and CD8+ T cells, CD19+ B cells and CD16/56+ NK cells), and analysis of lymphocyte proliferation responses (Sanchez-Ramon, et al. 2019). Humoral immunity assessment includes measurement of serum levels of immunoglobulin (Ig) A, M and G, IgG subclasses and antibody responses to vaccines (Sanchez-Ramon, et al. 2019).

2.2. Syndromic immunodeficiency

Some PID combine abnormalities of the immune system with defects in other organ systems and are thus designated "syndromic PID" (Bousfiha, et al. 2018, Kersseboom, et al. 2011). The associated features in syndromic PID include short stature, facial dysmorphism, neurologic abnormalities and skin disorders (Bousfiha, et al. 2018, Kersseboom, et al. 2011).

Syndromic PID with short-stature can be further subdivided according to the presence or absence of skeletal dysplasia. The examples of syndromic PID with growth failure and no skeletal dysplasia are, among others, DNA repair defects like Nijmegen breakage syndrome, Bloom syndrome, DNA ligase IV syndrome, DNA ligase 1 deficiency and cernunnos deficiency (Bousfiha, et al. 2018, Kersseboom, et al. 2011, Maffucci, et al. 2018).

PID associated with skeletal dysplasia include 1) cartilage-hair hypoplasia (MIM # 250250), 2) Schimke immuno-osseous dysplasia (MIM # 242900) (Schimke, et al. 1974), 3) Roifman syndrome (MIM # 616651) (Roifman 1999), 4) spondyloenchondrodysplasia with immune dysregulation (MIM # 607944) (Roifman and Melamed 2003), 5) immunodeficiency-23 (MIM # 615816) (Stray-Pedersen, et al. 2014), and 6) immunoskeletal dysplasia with

neurodevelopmental abnormalities (MIM # 617425) (Oud, et al. 2017). The underlying genetic defect, the type of skeletal dysplasia and immunodeficiency, and other associated features are distinct for each of these diseases (*Table 1*) (Baradaran-Heravi, et al. 2008, Briggs, et al. 2016, Dinur Schejter, et al. 2017, Guo, et al. 2017, Lipska-Zietkiewicz, et al. 2017, Notarangelo 2017, Pacheco-Cuellar, et al. 2017, Stray-Pedersen, et al. 2014).

Table 1. Primary immunodeficiency disorders associated with skeletal dysplasia.

Disease	**Genetic defect**	**Skeletal features**	**Immunodeficiency**	**Other features**
CHH	*RMRP*	Metaphyseal chondrodysplasia	Combined	Hair hypoplasia, anemia, increased incidence of malignancies and Hirschsprung disease
SIOD	*SMARCAL1*	Spondyloepiphyseal dysplasia	Combined	Glomerulopathy, abnormal skin pigmentation, dysmorphic features, cerebral ischemia, bone marrow hypoplasia
Roifman syndrome	*RNU4ATAC*	Spondyloepiphyseal dysplasia	Humoral	Retinal dystrophy, developmental delay, dysmorphic features, autoimmunity
SPENCDI	*ACP5*	Spondylometaphyseal dysplasia	Combined	Autoimmunity, developmental delay, spasticity, intracranial calcifications
IMD23	*PGM3*	Spondylometaphyseal dysplasia	Combined	Cardiovascular abnormalities, developmental delay, dysmorphic features
ISDNA	*EXTL3*	Spondyloepimetaphyseal dysplasia	Combined	Developmental delay, dysmorphic features, liver cysts

CHH cartilage-hair hypoplasia, IMD23 immunodeficiency-23, ISDNA immunoskeletal dysplasia with neurodevelopmental abnormalities, SIOD Schimke immuno-osseous dysplasia, SPENCDI spondyloenchondrodysplasia with immune defect.

2.3. Historical overview of cartilage-hair hypoplasia

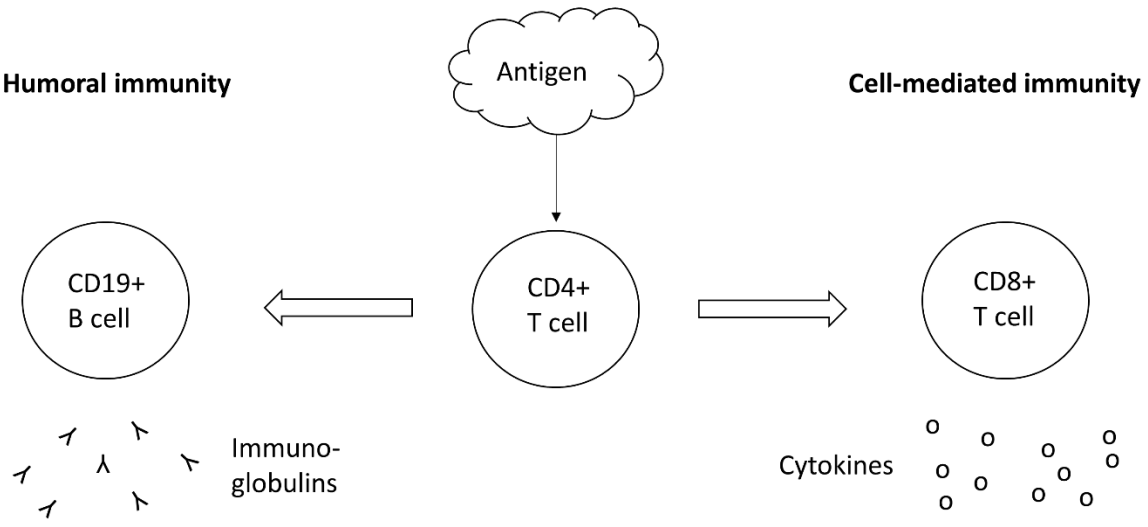
Maroteaux et al reported a specific form of metaphyseal dysplasia in 1963 (Maroteaux, et al. 1963). Two years later, McKusick et al described a cohort of 77 Amish patients with a similar type of growth failure (McKusick, et al. 1965). These patients presented with short-limbed short stature at birth and fine, sparse and light-colored hair. Additional features in some subjects included malabsorption, congenital megacolon and fatal varicella. Short stature was the consequence of cartilage hypoplasia confined to the metaphyseal parts of the long bones. The frequency of this type of skeletal dysplasia in the Amish population was estimated to be 1-2: 1,000 live births and the disease was named CHH.

In 1972, Perheentupa et al reported 21 patients with CHH from a single hospital in Finland, and this rare chondrodysplasia was then recognized to be overrepresented in the Finnish population (Perheentupa 1972). In 1990s Mäkitie et al described a large series of over 100 patients with CHH from Finland, demonstrating the incidence of 1: 23,000 live births. In addition to the short stature, most patients showed defective immunity, and more than half had increased susceptibility to infections (Makitie 1992, Makitie and Kaitila 1993). The association of CHH with anemia, malignancies and Hirschsprung disease was described and confirmed by later studies (Makitie, et al. 2001, Makitie, et al. 1992, Taskinen, et al. 2008).

In the 1970s, the laboratory features of immunodeficiency in CHH were reported in Amish and Finnish patients, highlighting lymphopenia, decreased numbers of B and/or T cells, impaired lymphocyte responses to mitogens and normal levels of serum immunoglobulins (Lux, et al. 1970, Ranki, et al. 1978, Virolainen, et al. 1978).

The mechanisms of immunodeficiency in CHH were uncovered in 1980s by demonstrating an intrinsic proliferation defect in lymphocytes and impaired T cell function (Pierce, et al. 1983, Pierce and Polmar 1982). CHH was then considered a pure T cell-mediated immunodeficiency with unaffected humoral immunity. However, cellular and humoral immunity are closely linked, and abnormalities in T cell compartment almost always affect the performance of B cells (*Figure 1*) (Noelle and Snow 1991). Consistent with this concept, the impairment of humoral immunity was confirmed in patients with CHH in 2000 (Makitie, et al. 2000).

Figure 1. A simplified graphic presentation of the interaction between humoral and cell-mediated immunity. Upon antigenic stimulation, CD4+ T cells participate in the activation of cytotoxic CD8+ T cells and antibody-producing B cells.



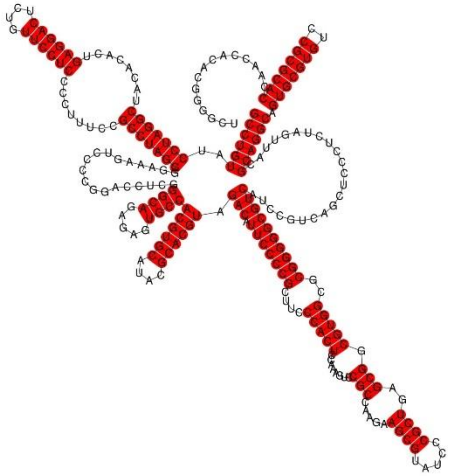
The genetic defect behind CHH remained a mystery for years until, in 2001, Ridanpää et al reported mutations in *RMRP* as a cause of CHH (Ridanpaa, et al. 2001). *RMRP* encodes the untranslated RNA component of the mitochondrial RNA-processing endoribonuclease which modulates multiple cellular functions, and this discovery opened the path for studies on CHH pathogenesis.

2.4. Pathogenesis of cartilage-hair hypoplasia

2.4.1. Mutations in the *RMRP* gene

The carrier frequency of CHH is Finland is approximately 1: 76, and all previously described Finnish patients with CHH share the same variant in *RMRP*, either in homozygous or compound heterozygous state (Makitie 1992). This ancient founder mutation n.71A>G (NCBI reference sequence: NR_003051.3, previously known as n.70A>G) is also the disease-causing variant in the Amish patients with CHH (Ridanpaa, et al. 2003, Ridanpaa, et al. 2002). Over 70 other *RMRP* variants have been described in subjects with CHH (Martin and Li 2007, Mattijssen, et al. 2010). Some mutations are situated in the promoter region and can affect the level of *RMRP* transcription, while others are located in all domains of the transcribed region, either affecting highly conserved nucleotides or altering the secondary structure of RMRP (*Figure 2*) (Thiel, et al. 2007).

Figure 2. The secondary structure of RMRP. The position of the most common disease-causing variant worldwide, n.71A>G, is marked by red circle. Modified with permission from fRNAdb © Toutai Mituyama (National Institute of Advanced Industrial Science and Technology) licensed under CC Attribution-Share Alike 4.0 International.

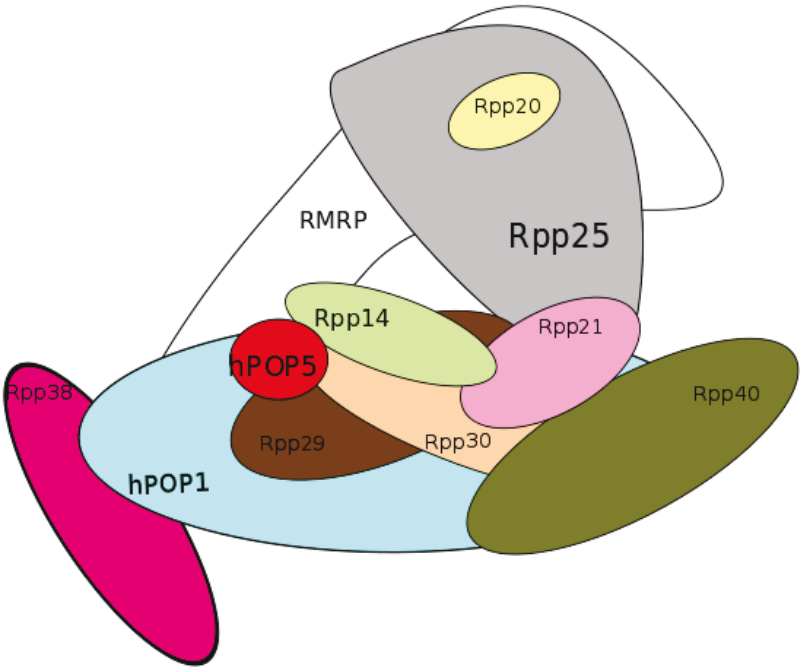


In addition to CHH, two other types of skeletal dysplasia are linked to *RMRP*, anauxetic dysplasia-1 (MIM # 607095), and metaphyseal dysplasia without hypotrichosis (MIM # 250460). Anauxetic dysplasia differs from CHH by an extremely severe short stature, the involvement of vertebrae, pelvis and femoral epiphyses, and the absence of other extra-skeletal manifestations except for mild mental retardation (Horn, et al. 2001, Menger, et al. 1996, Thiel, et al. 2005). Metaphyseal dysplasia without hypotrichosis refers to an isolated chondrodysplasia with no extra-skeletal features (Bonafe, et al. 2002, Verloes, et al. 1990).

2.4.2. Cellular consequences of the *RMRP* mutations

RMRP is the RNA component of the mitochondrial RNA-processing ribonuclease, forming this universal eukaryotic enzyme complex together with ten protein subunits (*Figure 3*) (Esakova and Krasilnikov 2010). Mutations in *POP1* gene encoding the POP1 subunit underlie anauxetic dysplasia-2 (MIM # 602486) (Barraza-Garcia, et al. 2017, Elalaoui, et al. 2016, Glazov, et al. 2011), highlighting the crucial importance of RNase MRP in skeletal development.

Figure 3. The structure of human mitochondrial RNA-processing ribonuclease, showing the 10 protein subunits and the one RNA component RMRP. Reproduced with permission from the RNApathways DB (http://www.genesilico.pl/rnapathwaysdb) (Milanowska, et al. 2013).



The molecular mechanisms contributing to immunodeficiency in CHH still remain incompletely understood. At the cellular level, the consequences of *RMRP* mutations in humans include impaired rRNA and mRNA processing which results in prolonged cell cycle (Thiel, et al. 2005). The degree of the RNA degradation defect has been reported to correlate with the severity of clinical features (Thiel, et al. 2007). rRNA and mRNA cleavage activity correlate with the degree of bone dysplasia and immunodeficiency, respectively (Thiel, et al. 2007). In addition, RMRP is the source of small RNAs that have regulating activity on genes associated with cell proliferation and differentiation (Rogler, et al. 2014).

The cellular basis of immunodeficiency in CHH includes reduced growth of granulocyte-macrophage progenitors from the bone marrow (Juvonen, et al. 1995), defective proliferation and increased apoptosis of the peripheral T cells (de la Fuente, et al. 2011), as well as decreased thymopoiesis and thymic dysplasia (Kavadas, et al. 2008).

2.4.3. Diseases of the non-coding RNAs

Only a few human diseases are caused by mutations in non-coding RNAs. These include CHH (*RMRP,* MIM # 157660), Roifman syndrome, Lowry-Wood syndrome and microcephalic osteodysplastic primordial dwarfism, type 1 (*RNU4ATAC*, MIM # 601428), and dyskeratosis congenita (*TERC,* MIM # 602322). While straightforward mechanisms explain clinical consequences of mutations in *RNU4ATAC* and *TERC*, this is not the case with *RMRP*.

RNU4ATAC mutations impair minor intron splicing, which affects about 800 genes associated with DNA repair, RNA processing and cell cycle progression (Merico, et al. 2015). Interestingly, in patients with *RNU4ATAC* mutations, some clinical manifestations overlap with those in CHH. Growth failure, skeletal dysplasia and immunodeficiency characterize both, Roifman syndrome and CHH. However, patients with Roifman syndrome also present with retinal dystrophy and cognitive delay, both absent in CHH, and their skeletal changes consist of spondyloepiphyseal dysplasia compared to metaphyseal chondrodysplasia of CHH (*Table 1*) (Merico, et al. 2015). In addition, the immunodeficiency in Roifman syndrome arises from decreased numbers of B cells and hypogammaglobulinemia, leaving T cells unaffected (Heremans, et al. 2018). This contrasts the combined immunodeficiency in CHH (*Table 1*). In microcephalic osteodysplastic primordial dwarfism, a striking similarity with CHH is the hair hypoplasia, as well as metaphyseal skeletal changes. Severe microcephaly, brain abnormalities, epilepsy, neuroendocrine dysfunction and facial dysmorphism distinguish microcephalic osteodysplastic primordial dwarfism from CHH (Kroigard, et al. 2016, Merico, et al. 2015). Lowry-Wood syndrome is an allelic condition similar to Roifman syndrome, but less severe. It is characterized by multiple epiphyseal dysplasia without spinal involvement, distinct from Roifman syndrome, microcephalic osteodysplastic primordial dwarfism and CHH

(Farach, et al. 2018). Interestingly, the characteristic feature of limited elbow extension in CHH has been also reported in individuals with Lowry-Wood syndrome (Shelihan, et al. 2018).

Patients with dyskeratosis congenita present with impaired skin pigmentation, mucosal and nail abnormalities, but also share some clinical manifestations with CHH, such as growth failure, immunodeficiency, anemia and increased incidence of malignancies (Nelson and Bertuch 2012, Savage 1993). Dyskeratosis congenita is a classic disorder of telomere biology, demonstrating the consequences of defective telomerase maintenance. *TERC* mutations affect telomerase activity leading to shorter telomeres (Vulliamy, et al. 2001). Telomeres are the end-fragments of human chromosomes that shorten with every cell division and are then repaired by telomerase (Harley, et al. 1990, Morin 1989). The telomerase complex consists of an RNA component *TERC*, a catalytic reverse transcriptase subunit TERT and associated proteins (Bertuch 2016). *RMRP* can bind TERT to form a distinct ribonucleoprotein complex that can produce double-stranded RNAs and regulate *RMRP* expression (Maida, et al. 2009). The impact of this *RMRP*-TERT interaction on telomeres in CHH has not been studied previously.

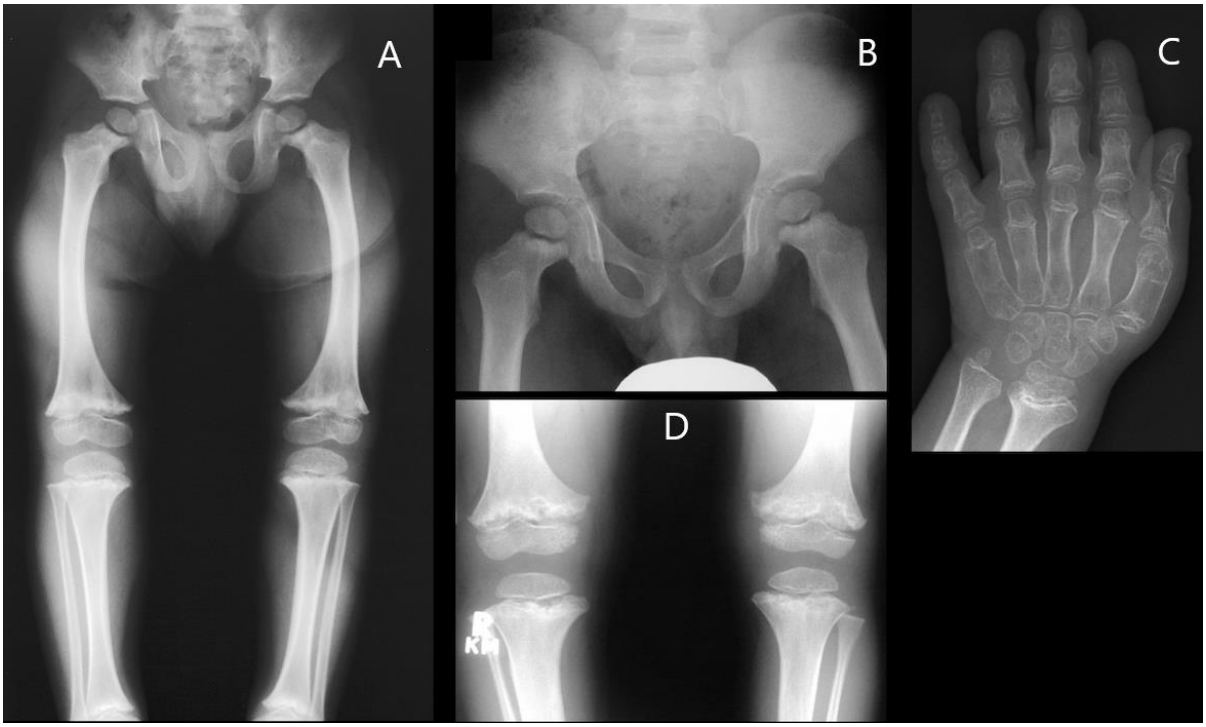
2.5. Skeletal features of cartilage-hair hypoplasia

The hypoplastic cartilage tissue confined mostly to the metaphyseal regions of long bones underlies the characteristic dysplastic skeletal phenotype in subjects with CHH (McKusick, et al. 1965). The onset of growth failure is prenatal and typical features of chondrodysplasia can be seen at birth in the majority of patients (Makitie, et al. 1992, McKusick, et al. 1965). The limbs are short, show signs of ligamentous laxity but limited elbow extension (Makitie and Kaitila 1993, McKusick, et al. 1965). Fingers and toes are short and flexible. Other manifestations include chest deformity, varus deformity of the lower extremities, increased lumbar lordosis and occasionally scoliosis (Makitie and Kaitila 1993, McKusick, et al. 1965).

Radiologic signs assisting the diagnosis are typically present before the closure of the epiphyses and are thus absent in adulthood (McKusick, et al. 1965). The classic appearance of the metaphyses of the long bones is seen most clearly in the knee and include flaring, scalloping, irregularity, sclerosis, fragmentation and cystic changes (*Figure 4*) (McKusick, et al. 1965, Riley, et al. 2015).

Birth length is subnormal in 70% of patients (Makitie and Kaitila 1993) and growth failure is progressive, especially during the first year of life and during puberty (Makitie, et al. 1992). The median adult height is 131 cm for males and 123 cm for females (Makitie, et al. 1992). Importantly, patients with CHH can present with normal height and no clinical or radiological signs of skeletal dysplasia (Kavadas, et al. 2008, Klemetti, et al. 2017).

Figure 4. Radiographs of patients with cartilage-hair hypoplasia at 4-6 years of age demonstrating the characteristic dysplastic appearance of the long bones with decreased length and widened and irregular metaphyses especially in the distal femur and proximal tibia (A, D), but also in the proximal femur (B), distal radius and metacarpals (C). The hand radiograph shows short tubular bones (C).



2.6. Extra-skeletal features of cartilage-hair hypoplasia

2.6.1. Infections

CHH patients with symptomatic immunodeficiency typically suffer from recurrent upper and lower respiratory tract infections (RTI), including otitis media, sinusitis and pneumonia (*Table 2*), caused by common pathogens like *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* (Bailly-Botuha, et al. 2008, Horn, et al. 2010). This pattern of infections apparently resembles humoral immunodeficiency, and a case of CHH with fatal enteroviral meningoencephalitis illustrates the severity of B cell dysfunction (Vatanavicharn, et al. 2010).

However, some patients with CHH manifest symptoms of predominantly T cell impairment and severe combined immunodeficiency (SCID) phenotype (Roifman, et al. 2006). They acquire opportunistic infections with *Pneumocystis jiroveci*, *Aspergillus spp*, herpes viruses and other typical SCID pathogens (*Table 2*). Interestingly, apart from severe varicella,

opportunistic infections have only rarely been described in Finnish patients with CHH compared to other cohorts (Makitie, et al. 2001).

Infections, mostly pneumonia and sepsis, are the main cause of death in Finnish children with CHH (Makitie and Kaitila 1993, Makitie, et al. 2001). However, not all patients suffer from recurrent or severe infections, despite obvious laboratory evidence for impaired immunity, including decreased CD3+, CD4+ and CD8+ T cells, CD19+ B cells, and recent thymic emigrants, as well as abnormal lymphocyte proliferation responses (de la Fuente, et al. 2011, Makitie and Kaitila 1993). In various cohorts, the prevalence of increased susceptibility to infections ranges from 11% to 100%, being most commonly reported at around 60% (*Table 2*).

Several infection-related issues remain insufficiently explored in CHH, including the important question of whether the pattern of infections in childhood can predict clinical course and guide management decisions. In addition, it is unknown, whether recurrent RTI in childhood correlate with the development of chronic lung disease and bronchiectasis.

2.6.2. Lung disease

Bronchiectasis is the permanent dilatation of the airway resulting from chronic inflammation and infection, and thus is an expected complication in patients suffering from recurrent RTI (Flume, et al. 2018). The prevalence of bronchiectasis in large PID cohorts ranges from 9% to 14% (Owayed and Al-Herz 2016, Rezaei, et al. 2006), and among various PID, patients with common variable immunodeficiency demonstrate the highest prevalence of bronchiectasis (57-70%) (Reisi, et al. 2017, Thickett, et al. 2002). In subjects with PID, low IgA is an independent risk factor for the development of bronchiectasis (Quinti, et al. 2011).

Patients with CHH can present with a range of pulmonary problems, including asthma, viral, bacterial and fungal infections, interstitial lung disease and bronchiectasis (Bordon, et al. 2010, de la Fuente, et al. 2011, Rider, et al. 2009). Bronchiectasis has been reported in children with CHH as young as 2.5 years, mostly in severe cases requiring HSCT (Bordon, et al. 2010, Moshous, et al. 2011). The high prevalence of bronchiectasis (52%) has been demonstrated in a retrospective cohort of 15 subjects with CHH and chronic respiratory symptoms (Toiviainen-Salo, et al. 2008). In this report, one patient required a lobectomy, and another died of pneumonia, underscoring the clinical significance of bronchiectasis. No clinical or laboratory features differed in patients with and without bronchiectasis, except for the absence of lymphopenia in the bronchiectasis group. Importantly, of the eight patients with bronchiectasis, only two demonstrated low IgG levels.

Plain chest radiographs are usually insufficient to diagnose bronchiectasis or interstitial lung changes, and therefore, high-resolution computed tomography (HRCT) should be performed if these conditions are suspected (Smevik 2000). However, radiation exposure is a major

concern in patients with PID, adding to the increased risk of cancer. Lung magnetic resonance imaging (MRI) could be a promising alternative as it has demonstrated a sufficient correlation with CT in patients with cystic fibrosis, primary ciliary dyskinesia and PID (Milito, et al. 2015, Montella, et al. 2012, Puderbach, et al. 2007, Sileo, et al. 2014). However, with the current techniques available, the sensitivity of MRI may be insufficient to identify subtle changes, especially in small peripheral bronchi (Biederer, et al. 2012).

Pulmonary changes in patients with CHH have not been examined systematically, and the role of lung MRI in assessing their lung pathology is unclear. The prevalence of bronchiectasis in clinically unselected patients with CHH and risk factors for the development of bronchiectasis remain unknown. Early recognition of bronchiectasis is of critical importance as these patients may develop rapid fatal infections (Horn, et al. 2010) and may require antimicrobial prophylaxis or immunoglobulin replacement therapy (IGRT) to combat progressive lung damage.

2.6.3. Malignancies

Patients with PID are more susceptible to malignancies, mostly non-Hodgkin's lymphoma (Mayor, et al. 2018, Mortaz, et al. 2016). Patients with CHH are not an exception, demonstrating a sevenfold risk of cancer, primarily non-Hodgkin's lymphoma (standardized incidence ratio (SIR) 90) and basal cell carcinoma (SIR 35) (Eisner and Russell 2006, Makitie, et al. 1999, Taskinen, et al. 2008).

Non-Hodgkin's lymphoma is a disease of young adults with CHH, given the SIR of 130 in the age group of 15-29 years (Taskinen, et al. 2008). It carries a poor prognosis in CHH cohort with median survival time of 6 months after the diagnosis (Taskinen, et al. 2008), and is the major cause of death in adults with CHH (Makitie, et al. 2001). This is similar to patients with other PID, who develop malignancies and have shorter survival compared with general population, as well as show high rates of relapse and secondary malignancy (Attarbaschi, et al. 2016). HSCT is considered a feasible therapeutic option in these individuals (Attarbaschi, et al. 2016, Wolska-Kusnierz, et al. 2015).

The pathogenesis of malignancy in CHH is incompletely understood and probably combines several pathways. Lymphoproliferative disorders in patients with CHH can be Epstein-Barr virus-driven in some (McCann, et al. 2014, Sathishkumar, et al. 2018, Taskinen, et al. 2013), but not all cases (Nguyen, et al. 2018). While Epstein-Barr virus induces lymphoma in patients with Wisckott-Aldrich syndrome, most cases of lymphoma in subjects with common variable immunodeficiency are Epstein-Barr virus-negative (Mortaz, et al. 2016). Therefore, not only impaired viral suppression, but other mechanisms, such as chromosomal instability, may play a role (Hauck, et al. 2018, Verhoeven, et al. 2018).

2.6.4. Autoimmune diseases

Autoimmune phenomena indicate immune dysregulation, and are, therefore, important signs of PID. Several reports describe patients with CHH and autoimmune diseases, such as enteropathy, hemolytic anemia, hypoparathyroidism, hypo- or hyperthyroidism, juvenile rheumatoid arthritis and neutropenia (Bacchetta, et al. 2009, Bonafe, et al. 2005, Bordon, et al. 2010, Rider, et al. 2009). Recently, a prevalence of 11% has been demonstrated for clinical autoimmunity in Finnish patients with CHH (Vakkilainen, et al. 2018). Despite frequent serum positivity for autoantibodies in individuals with CHH, no correlation with clinical symptoms has been observed (Biggs, et al. 2017, Vakkilainen, et al. 2018).

2.6.5. Laboratory features of immunodeficiency in cartilage-hair hypoplasia

Blood immunologic parameters in patients with CHH are highly variable, demonstrating no or mild abnormalities or severe impairment of both, cellular and humoral immunity (*Table 2*). The most consistent feature seen in the majority of patients (69-100%) is the decreased lymphocyte proliferation in response to mitogens (phytohemagglutinin). Also, in two series where T cell excision circles and recent thymic emigrants had been measured, they were low or absent in all tested samples. Many patients (36-94%) are lymphopenic, mostly due to the decreased numbers of T lymphocytes, especially CD4+ cells. CD16/56+ cell counts are usually normal but can be both elevated or decreased in some patients. The numbers of B cells are low in 9-75% of CHH cases, but hypogammaglobulinemia is infrequent. Instead, elevated IgG levels have been reported in 17-64% of patients.

Neutropenia has been described in 6-27% of patients with CHH and may contribute to the increased susceptibility to infections (Ammann, et al. 2004, Makitie and Kaitila 1993, Makitie, et al. 1998). Up to 79% of children with CHH manifest anemia, often megaloblastic, which usually is mild and resolves spontaneously (Kainulainen, et al. 2014, Makitie and Kaitila 1993). However, in approximately 6% of patients, anemia is severe and transfusion-dependent (Taskinen, et al. 2013).

To further complicate immunological evaluation, laboratory parameters in patients with CHH may fluctuate with time. Lymphopenia can be absent at presentation and develop later, lymphocyte proliferative responses can improve or worsen, and IgG levels can be decreased during infancy and then normalize, while IgM can be undetectable and then reappear (Kainulainen, et al. 2014, Kavadas, et al. 2008).

While immunoglobulin levels, lymphocyte proliferation responses and the numbers of CD3+, CD4+ and CD8+ T cells, CD19+ B cells and CD16/56+ NK cells have been studied previously in most of the published CHH case series (*Table 2*), some other immunologic parameters have been rarely or never reported. Paucity of data exists regarding T and B cell subpopulations

and specific antibody responses in individuals with CHH (de la Fuente, et al. 2011, Kainulainen, et al. 2014). Furthermore, previous studies have included mostly pediatric patients, and data on the immune function in adults with CHH remain scarce.

The clinical importance of impaired lymphocyte proliferative responses in subjects with CHH remain unclear. Responses to phytohemagglutinin are better in some patients with milder clinical course, including those with less infections (de la Fuente, et al. 2011, Kavadas, et al. 2008). In other reports, proliferative responses do not correlate with susceptibility to infections (Makitie and Kaitila 1993, Makitie, et al. 1998, Rider, et al. 2009). In CHH patients with more frequent RTI, both lower and higher absolute lymphocyte counts have been reported (Makitie, et al. 1998, Rider, et al. 2009), and, paradoxically, higher T cell and CD4+ cell counts and higher IgG levels (Makitie, et al. 1998, Makitie, et al. 2000). Two patients with CHH and CID necessitating HSCT showed elevated CD16/56+ counts, low IgG and undetectable IgA in the first 2 years of life, distinguishing them from the other 23 patients in the studied cohort (Rider, et al. 2009), but these correlations have not been confirmed in other studies. Altogether, previous reports provide ambivalent correlations of laboratory parameters with clinical course.

2.7. Variability of manifestations

The variability of clinical features in CHH has been well described, even in patients with identical *RMRP* mutations and within families (Kavadas, et al. 2008, Makitie and Kaitila 1993, Rider, et al. 2009, van der Burgt, et al. 1991). All previously reported Finnish patients with CHH were either homozygous or heterozygous for the most prevalent *RMRP* mutation n.71A>G, and therefore, phenotype variations are considered to be independent of the genotype. Polymorphisms in *RMRP* gene and non-allelic modifiers have been suggested as possible mechanisms (Notarangelo, et al. 2008, Thiel, et al. 2007). Previous studies have failed to explain the heterogeneity of symptoms or provide prognostic factors for the development of severe immunodeficiency or malignancies.

The degree of immunodeficiency in CHH ranges from completely asymptomatic to severe combined immunodeficiency necessitating HSCT. Some patients present with recurrent or severe infections in the first year of life, while others develop late-onset immunodeficiency as adults (Horn, et al. 2010, Rider, et al. 2009). Such a diversity of clinical manifestations complicates the management of CHH on the individual level. While HSCT is the only life-saving option for severe cases, the optimal management of other patients remains uncertain and data on clinical and laboratory factors associated with prognosis are urgently needed.

Table 2. Infections and laboratory features in patients with cartilage-hair hypoplasia.

Study	**N**	**Age of pt, yrs**	**Definition of increased susceptibility to infections**	**Prevalence of increased susceptibility to infections, N (%)**	**Types of infections**	**Immunoglobulin levels, N (%)**	**Cell counts, N (%)**	**Abnormal lymphocyte proliferative responses to PHA, N (%)**
Makitie and Kaitila (1993)	108	0.8 – 52	>6 uncomplicated URTI or ≥3 protracted purulent inf such as OM or Sin in the preceding yr	58/103 (56)	OM, Pn, sepsis, severe varicella, Sin, URTI	NA	Ly ↓ 51/79 (65) Neutropenia 21/79 (27)	53/60 (88)
Makitie, et al. (1998)	35	0.1 – 55.5	>6 uncomplicated URTI or ≥3 protracted purulent inf such as OM, Sin or Pn per yr	11/35 (11)	OM, Pn, Sin, URTI	Normal 16/16 (100)	Ly ↓ 12/33 (36) Neutropenia 2/33 (6) CD4+ ↓ 17/30 (57) CD8+ ↓ 8/30 (27) ↑ 1/30 (3) CD19+ ↓ 2/23 (9) ↑ 2/23 (9)	22/32 (69)
Makitie, et al. (2000)	20	1.7 – 16.7	>6 uncomplicated URTI or ≥3 protracted purulent inf such as OM, Sin or Pn in the preceding yr	10/20 (50)	RTI	IgG ↓ 0/20 (0) IgG ↑ 5/20 (25) IgM normal 20/20 (100) IgA deficiency 2/20 (10)	NA	NA
Guggenheim, et al. (2006)	3	6 – 21	Omenn syndrome described in 2 pts	2/3 (67)	Pn (PJ), thrush	IgG ↓ 1/3 (33) IgM ↓ 1/3 (33) IgA deficiency 3/3 (100)	CD3+ ↓ 3/3 (100) CD4+ ↓ 3/3 (100) CD8+ ↓ 3/3 (100) NK ↓ 1/3 (33)	3/3 (100)
Hermanns, et al. (2006)	27	0.6 – 32	Inf incidence significantly above average in age-matched controls	12/22 (55)	RTI or other	Ig-s ↓ 2/5 (40)	CD3+ ↓ 6/9 (67)	2/2 (100)

Adeno adenovirus, bact bacterial, CD cluster of differentiation, CD19+ B cells, CD3+ T cells, CD4+ helper T cells, CD8+ effector T cells, CMV cytomegalovirus, EBV Epstein-Barr virus, EBV-LP EBV-related lymphoproliferation, HHV-6 human herpes virus 6, HSCT hematopoietic stem cell transplantation, HSV herpes simplex virus, Ig immunoglobulin, inf infection, Ly lymphocytes, N number of patients, NA not available, NK natural killer cells, OM otitis media, PHA phytohemagglutinin, PJ *Pneumocystis jiroveci,* Pn pneumonia, pt patients, Sin sinusitis, TREC T cell receptor excision circles, URTI upper respiratory tract infection, yr year, ↓ decreased, ↑ increased.

Table 2 (continued). Infections and laboratory features in patients with cartilage-hair hypoplasia.

Study	**N**	**Age of pt, yrs**	**Definition of increased susceptibility to infections**	**Prevalence of increased susceptibility to infections, N (%)**	**Types of infections**	**Immunoglobulin levels, N (%)**	**Cell counts and TREC, N (%)**	**Abnormal lymphocyte proliferative responses to PHA, N (%)**
Kavadas, et al. (2008)	12	0.2-6, 44	Inf suggestive of immune deficiency	8/12 (67)	OM, Pn (*Aspergillus,* CMV, PJ), thrush	IgG ↓ 3/11 (27) IgG ↑ 7/11 (64) IgM ↓ 1/11 (9) IgA deficiency 3/11 (27)	Ly ↓ 10/12 (83) CD3+ ↓ 12/12 (100) CD8+ ↓ 12/12 (100) CD4+ ↓ 11/12 (92) CD19+ ↓ 6/12 (50) NK ↓ 2/12 (17) TREC ↓ 4/5 (80)	11/11 (100)
Rider, et al. (2009)	25	0.8-21	Life-threatening inf prior to age 2 yrs or >2 bact inf / yr during the first 2 yrs of life	8/25 (32)	Disseminated HSV, parvovirus; meningitis (*Haemophilus influenzae*); OM; Pn (bact, CMV); sepsis; Sin; thrush	IgG ↓ or ↑ in some IgM ↓ in some IgA deficiency 3/25 (12)	Ly ↓ in the majority	In most
Bordon, et al. (2010)	16	0.7-19	All pts required HSCT	16/16 (100)	Adeno enteritis and hepatitis; disseminated CMV, EBV, HSV; OM; Pn (bact, varicella-zoster virus); severe varicella	IgG ↓ 5/16 (31) IgM ↓ 5/16 (31) IgA ↓ 8/16 (50)	Ly ↓ 15/16 (94) CD3+ ↓ 15/16 (94) CD4+ ↓ 15/16 (94) CD8+ ↓ 15/16 (94) CD19+ ↓ 12/16 (75) NK ↓ 2/13 (15)	14/15 (93)
de la Fuente, et al. (2011)	18	1-21	Severe and/or recurrent inf, not further defined	11/18 (61)	EBV-LP, molluscum, parvovirus, Pn (*Aspergillus*, CMV), sepsis, URTI	Normal Ig-s 11/14 (79)	CD3+ ↓ 10/18 (56) CD4+ ↓ 18/18 (100) CD8+ ↓ 13/18 (72) CD19+ ↓ 8/18 (44) RTE ↓ 18/18 (100)	11/13 (85)
Ip, et al. (2015)	13	0-9	All pts required HSCT	13/13 (100)	Disseminated adeno, CMV, EBV, HHV-6; EBV-LP; enteritis (adeno, HHV-6); OM; RTI; severe varicella	IgG ↓ 4/12 (33) IgG ↑ 2/12 (17) IgA ↓ 7/12 (58) IgM ↓ 5/12 (42)	CD4+ ↓ 13/13 (100) CD8+ ↓ 9/13 (69) CD19+ ↓ 5/13 (38) TREC ↓ 9/9 (100)	9/11 (82)

3. AIMS OF THE STUDY

Several aspects of CHH remain poorly explored, including the pathogenesis, lung disease, detailed characteristics of immunologic phenotype, correlations of clinical and laboratory features, disease course and prognosis, as well as factors associated with adverse outcomes. To address these questions, we recruited and carefully examined a large cohort of Finnish children and adults with CHH aiming for sufficient sample size, detailed and accurate data collection and long-term follow-up.

The aims of this doctoral work were as follows:

1. To further explore the molecular consequences of *RMRP* mutations and the pathogenesis of CHH by measuring relative telomere length in a large cohort of patients with CHH, their first-degree relatives and healthy controls, and by analyzing the correlation of telomere length with the clinical and laboratory manifestations of CHH.

2. To further elucidate the immunologic features and determinants predicting clinical infections by studying infectious manifestations and performing a thorough immunologic characterization in a large group of children and adults with CHH.

3. To examine the prevalence of bronchiectasis, as well as clinical and immunologic risk factors for the development of bronchiectasis in a random sample of patients with CHH and compare the performance of lung MRI and HRCT in the assessment of lung changes.

4. To follow-up a large cohort of patients with CHH and analyze the clinical course and factors influencing survival and the development of malignancies.

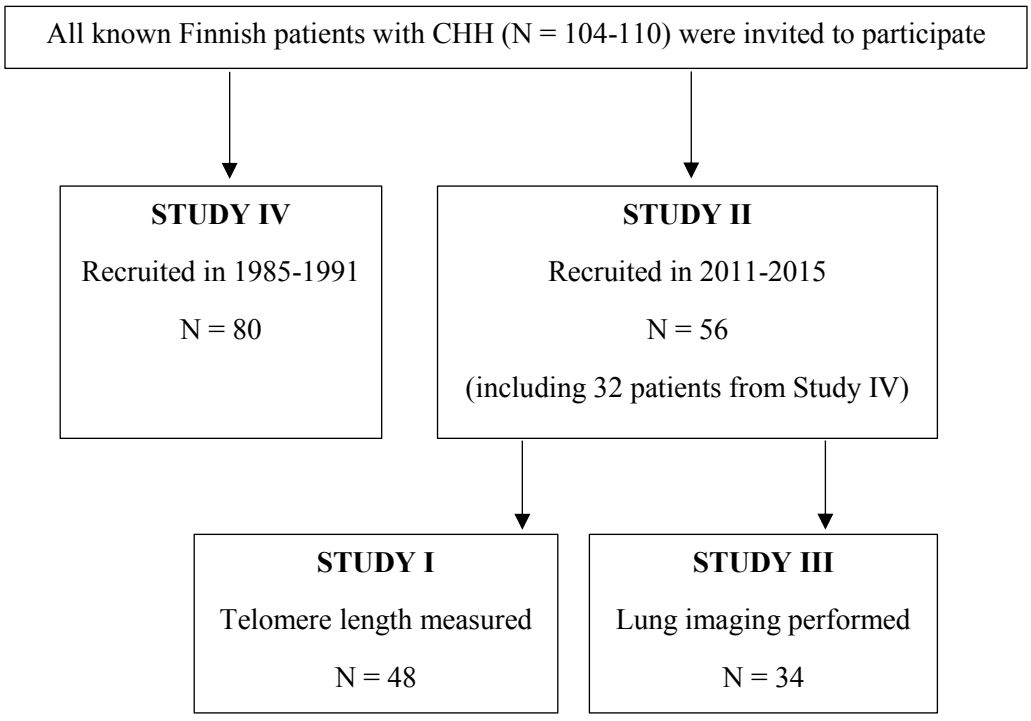
4. PATIENTS AND METHODS

4.1. Patients

The protocol for all studies was approved by the Institutional Research Ethics Committee at Children's Hospital, Helsinki University Hospital, Finland. All participants and/or their parents gave informed consent.

We used the Finnish Chondrodysplasia Registry to invite Finnish patients with genetically confirmed CHH (*Figure 5*). Regardless of their medical history, all subjects who agreed to participate were included. For study I, patients' first-degree relatives were contacted via the index persons.

Figure 5. Number (N) of recruited patients with cartilage-hair hypoplasia (CHH).

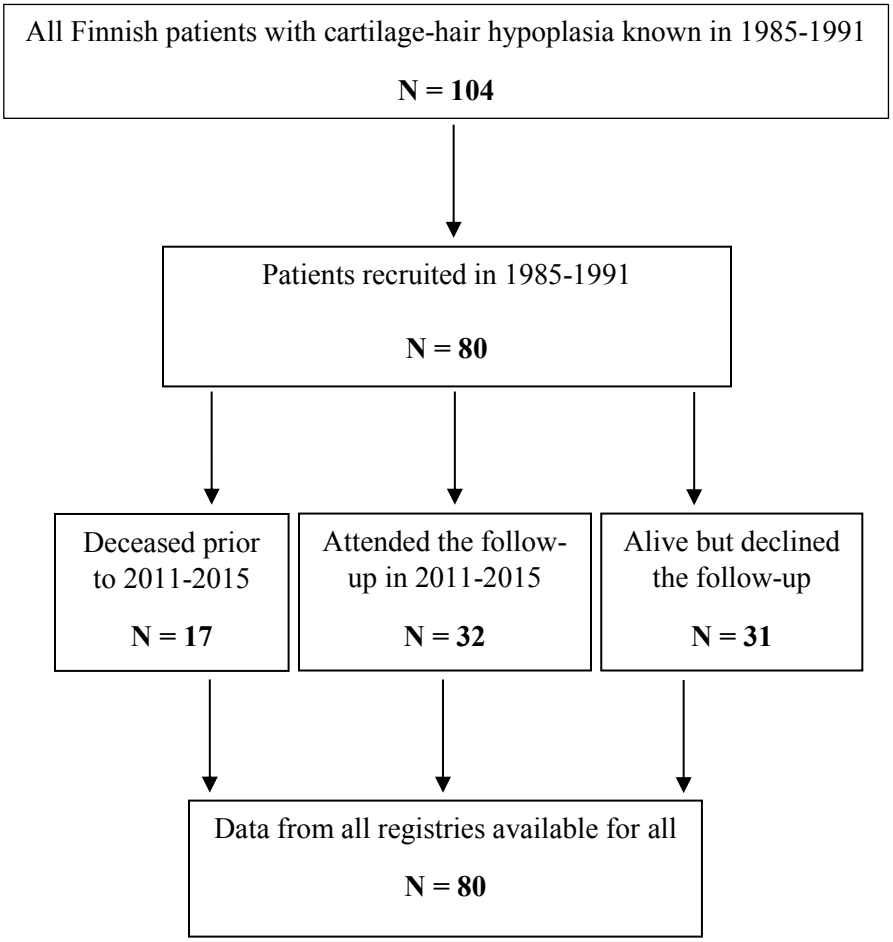


For study I, we used a control group of healthy *RMRP* mutation-negative individuals to compare the results of telomere measurements. We analyzed samples from 86 first-degree relatives of the patients, all unaffected, including 37 parents, 38 siblings and 11 children. Genetically, 74 of them were confirmed to be heterozygous carriers of *RMRP* mutations, while 12 lacked *RMRP* mutations and were included in the control group. Additionally,

samples from participants of our previous studies involving healthy children and adults (n = 94) were included as controls. Some statistical analyses implicated a case-control setting, for which age- and sex-matched healthy controls were identified for each patient or *RMRP* mutation carrier with age difference of no more than 12 months.

For Study IV, we recruited all known Finnish patients with CHH in 1985-1991 and followed them up in 2011-2015 (*Figure 6*).

Figure 6. Recruitment and follow-up of the patients for Study IV, and data availability.
N = number of patients.



4.2. Clinical data

During 1985-1991 and 2011-2015, study participants visited Helsinki University Hospital. We performed a standard clinical examination, interviewed patients for medical history and retrieved additional information from hospital records. In 2011-2015, a structured questionnaire inquired about hospitalizations, medications, surgical procedures, previous radiologic investigations, infections and respiratory symptoms. In addition, we recorded factors potentially influencing telomere length, including the history of smoking, intake of hormonal or immunosuppressive medications and obesity (body mass index Z-score). Clinical information available on healthy controls in Study I consisted of data on age, sex, ethnic background (all Finnish) and overall health (all healthy).

For all 80 patients from Study IV, we collected health information from the two Finnish National Medical Databases. Data from the Finnish National Care Registry for Health Care (Hilmo) covered the period from 1969 to 2016 and included data on inpatient health service providers, while the Finnish National Registry of Primary Health Care Visits (Avohilmo) covered outpatient health service provider data in 2011-2016. Information included dates of visits, diagnosed conditions, as well as diagnostic and therapeutic procedures. We then obtained all patients' health records from all identified inpatient health service providers for further analysis. In addition, we collected data on malignancies from the Finnish Cancer Registry, covering time period from 1953 to 2016, as well as mortality data from the Cause-of-death Registry of Statistics Finland for the period of 1971-2016. We analyzed the causes of death based on the registry data and also from the patient records.

We classified patients as having mild (height above the 75^{th} percentile for age and sex on the CHH growth curves), moderate (between 25^{th} and 75^{th} percentile) or severe (below the 25^{th} percentile) growth failure in accordance with age- and sex-specific growth data for patients with CHH based on the latest available height measurement (Makitie, et al. 1998, Makitie, et al. 1992). We also used birth length standard deviation (SD) score to characterize the severity of growth failure (Pihkala, et al. 1989). Patients were considered to have contracted varicella zoster virus if they had a history of varicella or detectable serum antibodies to varicella zoster virus, and we used hospitalization as a marker of severe varicella. Other definitions used in the studies are listed in *Table 3*.

Based on their clinical manifestations, patients were grouped as having: 1) no clinical symptoms of immunodeficiency, 2) clinical features of humoral immunodeficiency only and 3) clinical features of CID. The categorization differed for Studies I-II and Study IV due to the different settings and the availability of considerable amount of additional data in Study IV (*Table 3*). In this thesis, the definitions used in Study IV were applied.

For Study IV, we selected several primary outcomes: 1) mortality (assessed by evaluating deaths related to immunodeficiency, including death from infections, pulmonary diseases and malignancies), 2) the development of lymphoma and 3) the development of skin cancer.

Table 3. Terms and their definitions applied in the studies.

Term	*Definition*	
Children	Individuals aged 0-18.0 years	
Adults	Individuals aged over 18.0 years	
Chronic cough	Ongoing daily cough lasting at least one year	
Recurrent pneumonia	Two or more episodes within one year or three or more episodes during lifetime	
Recurrent otitis media and/or rhinosinusitis	Three or more episodes within six months, four or more episodes within a year or at least ten episodes during lifetime	
Sepsis	Compatible clinical signs presenting simultaneously with positive blood cultures	
Bacterial skin infections	Impetigo, boils and/or cellulitis	
Viral skin infections	Warts, molluscum contagiosum and/or recurrent mucocutaneous herpes simplex virus infections	
Refractory warts	Skin and/or anogenital warts persisting for years and unresponsive to multiple therapies	
Mucocutaneous Candida infections	Thrush beyond the first six months of life and esophagitis	
Opportunistic infections	Refractory warts, recurrent mucocutaneous herpes simplex virus infections, mucocutaneous Candida infections and/or severe varicella	
Humoral immunodeficiency	Study I, II	Otitis media or rhinosinusitis requiring surgery, sepsis, pneumonia and/or bronchiectasis
	Study IV	Recurrent respiratory tract infections and/or sepsis
Combined immunodeficiency	Study I, II	Additional features of refractory warts, recurrent and/or severe herpes virus infections, malignancy and/or autoimmunity
	Study IV	Additional features of autoimmunity and/or opportunistic infections

4.3. Laboratory data

To assess the immunologic phenotype, we obtained non-fasting blood tests from patients (n = 56) at the time of visits. In the majority of the patients (n = 48), as well as for their first-degree relatives, we also collected blood samples for DNA extraction and relative telomere length (RTL) measurement, while previously available DNA samples were used for healthy controls. DNA extraction was performed using 5 Prime Archive Pure DNA Blood kit (5 Prime GmbH, Hilden; Germany).

We analyzed leukocytes, neutrophils, eosinophils and lymphocyte subsets with flow cytometry and compared the results with local laboratory reference values. Previously published reference data were applied for cell counts in children (Shearer, et al. 2003), for CD3/CD4/CD45RA/CD31+ cells (recent thymic emigrants) (Boldt, et al. 2014, Schatorje, et al. 2012), for CD27+ (memory) B cells (Driessen, et al. 2011) and for other B and T cell subpopulations, including naive, activated and memory B and T cells, regulatory and double-negative T cells, as well as transitional and marginal zone-like B cells and plasmablasts (Boldt, et al. 2014, Wehr, et al. 2008).

We measured immunoglobulins and IgG subclasses as described previously (Makitie, et al. 2000) and applied the available reference values for IgG subclasses (Vlug, et al. 1994). Antibodies to tetanus toxoid (n = 43) and varicella zoster virus were measured with enzyme immunoassay (Aalto, et al. 1998, Jaaskelainen, et al. 2014, Kristiansen, et al. 1997). Concentrations of tetanus antibodies higher than 0.1 IU/ml were considered protective. We excluded patients receiving IGRT at the time of the studies I-III (n = 4) from analysis of antibodies to vaccines, IgG and subclasses.

In eight patients aged 23-67 years, we used fluoroimmunoassay to assess antibody responses to pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F after immunization with unconjugated 23-valent pneumococcal polysaccharide vaccine (Pneumovax®) from samples pre- and 3-8 weeks post-immunization (Timby, et al. 2015). We defined normal response as post-immunization antibody levels of ≥ 0.35 µg/ml and a fourfold rise in serotype-specific antibodies to ≥ 70% of serotypes (Sorensen and Leiva 2014).

In Study IV we also evaluated laboratory data from the hospital records and applied the reference values of the laboratories where the samples were analyzed.

4.3.1. *RMRP* gene sequencing

For the majority of patients (51/56, 80/80), genetic data was readily available. In addition, we sequenced *RMRP* in all samples to confirm the genotype in patients from study I and to detect heterozygous mutations in the patients' relatives and healthy controls. Primers for *RMRP* (GRCh37/hg19) were designed with Primer3 v.0.4.0 (http://frodo.wi.mit.edu/primer3/) tool, with a minimum of 60 bases of flanking regions adjacent to the coding region. PCR was amplified with DreamTaq (ThermoScientific, Waltham, MA, USA). DNA fragments were visualized on agarose gel with Midon Green Advanced DNA Stain (NIPPON Genetics, GmbH, Europe), purified with ExoSAP (USB, Cleveland, OH, USA) and labeled with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Following bidirectional sequencing with an ABI3730 sequencer (Applied Biosystems), chromatograms were analyzed with Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) using genomic NG_017041.1 and RNA reference sequence NR_003051.3.

4.3.2. Measurement of telomere length

Telomere length was measured with quantitative-PCR method (Cawthon 2002, Degerman, et al. 2014). DNA was analyzed twice in triplicate wells in a separate Telomere (TEL) and a single copy gene (HBG) reaction using the ABI7900HT instrument (Applied Biosystems). TEL/HBG (T/S) values were calculated with the $2^{-\Delta Ct}$ method, where ΔCt = average CtTEL - average CtHBG. RTL values were generated by dividing samples T/S value with the T/S value of a reference DNA of CCRF-CEM cell line, which was included in all runs. We categorized individuals as having short, average or long RTL for age based on the data from healthy controls. Subjects with RTL > 0.5 SD from the regression line for age *vs* RTL were considered to have long RTL, those with RTL < -0.5 SD – short RTL, and all the rest - average RTL. In further analysis, we used both, RTL itself and RTL categories.

4.4. Imaging studies

All patients from Study III (n = 34) underwent lung HRCT, and in 16 out of these 34 patients, lung MRI was performed on the same day. Two experienced radiologists who were blinded for the patients' clinical data together analysed and scored all MRI and HRCT images. Scoring was performed using modified Helbich (Bhalla) system (Puderbach, et al. 2007); the final scores in each patient and study were reached by consensus. Nine parameters were evaluated with a maximum possible score of 27 points (Puderbach, et al. 2007). A cut-off value of ≥7 points was chosen for the diagnosis of bronchiectasis.

4.5. Statistical analyses

Correlation of the tested laboratory parameters with clinical manifestations and primary outcomes were analyzed using the Fisher's exact test, the Mann-Whitney or the Kruskall-Wallis tests and regression analyses, as appropriate.

We compared the performance of HRCT and MRI by calculating the Spearman's rank correlation coefficient (rho). The latter parameter was also implicated to evaluate the influence of age on various laboratory parameters.

For the calculation of standardized mortality ratios (SMR), we derived the expected numbers of deaths by multiplying person-years by the mortality rate in general population. SMR were then calculated as the proportion of the number of observed and expected deaths. SIR for malignancies were derived by dividing the number of observed malignancy cases by the number of expected malignancies in general population. We evaluated the significance of difference between SMRs in subgroups of patients by SMR/SMR ratio and its 95%CI.

P values <0.05 were considered significant. Statistical analyses were accomplished with the IBM SPSS Statistics (versions 22-23).

5. RESULTS

5.1. Patient characteristics

5.1.1. General characteristics

Table 4 compares general and clinical characteristics of the study patients. Number of female patients outweighed the number of males in all studies. Patients from all age groups, from childhood to adulthood, were included. In the *RMRP* genotyping, all patients were either homozygous for the most common CHH causing variant n.71A>G, or compound heterozygous for n.71A>G/g.262G>T or n.71A>G and a 10-nucleotide duplication at position -13 (TACTCTGTGA). In Study IV, the surviving patients were followed-up for the median of 29.2 years (range 25.6 – 31.0 years).

Table 4. Characteristics of the study patients.

	Study I	**Study II**	**Study III**	**Study IV**
Focus of the study	Telomere length	Immunologic characteristics	Lung disease	Disease course and risk factors
General characteristics of the patients				
Number of patients	48	56	34	80
Number of males / females	17 / 31	19 / 37	6 / 28	35 / 45
Median age at recruitment (range), years	38 (6 – 70)	34 (0.7 – 68)	39 (13 – 68)	15 (0.0 – 50)
Homo-/heterozygous for *RMRP* n.71A>G variants	75% / 25%	77% / 33%	85% / 15%	78% / 22%
Prevalence of clinical features during lifetime, number (%)				
Otitis media	34/47 (72)	41/56 (73)	25/34 (74)	58/80 (73)
Rhinosinusitis	27/47 (57)	33/56 (59)	24/34 (71)	48/80 (60)
Pneumonia	10/47 (19)	11/56 (20)	8/34 (24)	32/80 (40)
Sepsis	NA	3/56 (5)	NA	8/80 (10)
Warts	15/47 (32)	19/56 (34)	NA	26/80 (33)
Mucocutaneous herpes simplex virus infections	NA	2/56 (4)	NA	2/80 (3)
Varicella requiring hospitalization	5/34 (15)	5/42 (12)	NA	4/66 (6)
Allergic rhinitis	NA	23/56 (41)	15/34 (44)	NA
Physician-diagnosed asthma	NA	NA	10/34 (29)	17/80 (21)
Malignancies	9/47 (19)	9/56 (16)	NA	21/80 (31)

NA not available, *RMRP* RNA component of the mitochondrial RNA-processing endoribonuclease

5.1.2. Infectious manifestations (Study IV)

The most common infections occurring in the study patients were RTI, characteristically ear infections in childhood and rhinosinusitis in teenagers and adults (*Table 4*). Although the prevalence of pneumonia, rhinosinusitis and otitis media was significant in the study cohort (40%, 60% and 73% respectively), only 38% of patients had recurrences of these infections. Common pathogens had been detected in the aspirates from middle ear and paranasal sinuses in patients with recurrent otitis media and rhinosinusitis respectively: *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*.

Other described infections included sepsis, skin infections and severe varicella (*Table 4*). Sepsis was more common in children (n = 7) than in adults (n = 2). Sepsis episodes in three children and both cases of sepsis in adults were associated with central venous catheter. Organisms causing sepsis included *Candida spp*, *Enterococcus spp*, *Haemophilus influenzae*, *Klebsiella pneumonia*, *Lactobacillus rhamnosus* and *Staphylococcus spp*. Viral skin infections, mostly warts, were more prevalent that bacterial skin infections (30/80, 38% *vs* 18/80, 23%). A significant proportion of patients with warts had refractory disease (9/22, 41%), all in adulthood.

Opportunistic infections were diagnosed in 20% of the patients and included mucocutaneous *Candida spp* infections, refractory warts and recurrent mucocutaneous herpes simplex virus infections, as well as severe varicella.

5.2. Clinical patterns and the course of immunodeficiency (Study IV)

Several patterns of the disease course were recognized. A large group of patients (46/80, 57%) had clinically asymptomatic immunodeficiency. However, eight of these 46 individuals developed non-skin malignancy of which five died. Clinical humoral immunodeficiency was demonstrated in 15 patients (15/80, 19%) and another 19 subjects developed clinical features of CID (19/80, 24%).

Importantly, clinical manifestations of immunodeficiency progressed in a significant proportion of patients (17/79, 22%). Six asymptomatic children developed immunodeficiency in adulthood. Eleven children with humoral immunodeficiency progressed to CID as adults. *Table 5* describes the course of the immunodeficiency and the outcomes. The immunodeficiency-related mortality in patients grouped by the type of clinical immunodeficiency in childhood is illustrated in *Table 5*. Children with clinical features of CID (n = 7) demonstrated the most severe disease course, with only one patient being alive and cancer-free at the end of the follow-up. Of the 52 asymptomatic children and of 20 subjects with symptoms of humoral immunodeficiency in childhood, only 34 and five, respectively, were alive and had not developed cancer nor CID.

Table 5. The course of clinical immunodeficiency (ID) during lifetime in 80 patients with cartilage-hair hypoplasia. Malignancies are described as the total number of diagnosed cases of non-skin cancers.

Childhood		**Adulthood**		**Mortality and the development of malignancies, [mean age at cancer diagnosis]**	
No ID	52/80 (65%)	No ID	46/52 (88%)	Alive/Deceased	39 (85%) / 7 (15%)
				Cancer	9/46 (20%) **[42 years]**
		Humoral ID	5/52 (10%)	Alive/Deceased	5 (100%) / 0 (0%)
		Combined ID	1/52 (2%)	Alive/Deceased	0 (0%) / 1 (100%)
				Cancer	1/1 (100%) **[69 years]**
Humoral ID	20/80 (25%) 1/20 deceased	No ID	5/20 (25%)	Alive/Deceased	4 (80%) / 1 (20%)
				Cancer	1/5 (20%) **[26 years]**
		Humoral ID	3/20 (15%)	Alive/Deceased	2 (67%) / 1 (33%)
		Combined ID	11/20 (55%)	Alive/Deceased	8 (73%) / 3 (27%)
				Cancer	2/11 (18%) **[39 years]**
Combined ID	7/80 (9%) 1/7 deseased	No ID	1/7 (14%)	Alive/Deceased	1 (100%) / 0 (0%)
		Combined ID	5/7 (72%)	Alive/Deceased	2 (40%) / 3 (60%)
				Cancer	4/5 (80%) **[23 years]**
Insufficient data	1/80 (1%)	Humoral ID	1/1 (100%)	Alive/Deceased	0 (0%) / 1 (100%)

5.3. Laboratory immunologic characteristics (Study II)

Table 6 describes immunologic laboratory parameters in a cohort of patients with CHH. Lymphopenia was detected in 55% of the study subjects. Decreased B cell counts were reported more frequently than decreased T cell or NK cell counts (67% *vs* 45% *vs* 7%). IgG level was low in a single patient, aged 58 years, who suffered from recurrent rhinosinusitis only. Naive thymic CD3+CD4+CD45RA+CD31+ cells were low in 27/52 patients (52%). Abnormalities detected in other T cell subpopulations (in n = 11, *Table 7*) consisted of 1) decreased naive CD4+ and CD8+ cells; 2) increased activated CD4+ cells; 3) increased central memory CD4+ and effector memory CD8+ cells; 4) increased TCR-α/β+ and decreased TCR-γ/δ+ cells. Regulatory T cells appeared normal in 10 out of 11 tested patients.

Table 6. Laboratory immunologic parameters in patients with cartilage-hair hypoplasia. Cell counts are reported as cells x10^9/l and immunoglobulin (Ig) levels as g/l.

	Normal values*	Number tested	Median (range)	Decreased in, number (%)	Increased in, number (%)
Leukocytes	3.4-8.2	56	5.65 (1.2-12.0)	7 (13)	7 (13)
Neutrophils	1.5-6.7	56	3.68 (0.28-8.4)	4 (7)	5 (9)
Lymphocytes	1.3-3.6	56	1.31 (0.26-3.84)	31 (55)	0 (0)
CD3+	0.85-2.28	55	0.92 (0.16-4.45)	25 (45)	2 (4)
CD4+	0.458-1.406	55	0.55 (0.12-3.83)	24 (44)	0 (0)
CD3+CD4+CD45RA+CD31+	0.024-0.824	52	0.03 (0.00-0.56)	27 (52)	0 (0)
CD8+	0.24-0.98	55	0.28 (0.04-1.72)	25 (45)	2 (4)
CD19+	0.12-0.43	55	0.12 (0.00-1.29)	37 (67)	0 (0)
CD27+IgD+	0.009-0.088	51	0.008 (0.000-0.044)	29 (57)	0 (0)
CD27+IgD-	0.013-0.122	51	0.098 (0.000-0.064)	34 (67)	0 (0)
CD16/56+	0.08-0.57	55	0.19 (0.05-0.63)	4 (7)	0 (0)
IgG	6.8-15.0	50	10.45 (4.20-15.90)	1 (2)	2 (4)
IgG1	4.9-11.4	50	8.25 (3.24-14.00)	1 (2)	0 (0)
IgG2	1.50-6.40	50	1.89 (0.27-4.56)	13 (26)	0 (0)
IgG3	0.20-1.63	50	0.44 (0.07-1.20)	8 (16)	0 (0)
IgG4	0.08-1.40	55	0.09 (0.00-0.92)	23 (46)	0 (0)
IgA	0.52-4.84	55	1.83 (0.00-7.49)	2 (4)	3 (5)
IgM	0.36-2.84	55	0.90 (0.20-3.06)	7 (13)	1 (2)

* Normal values in adults. In children age-specific normative data were used.

All 11 patients showed an increased proportion of activated CD21low, CD38low B cells (*Table 7*). When absolute numbers of B cell subpopulations were assessed, 7/11 patients had low naive B cells and low transitional cells and 6/11 patients had low marginal-zone like B cells. CD27+ B cell counts were decreased in the majority of patients.

Inadequate serotype-specific responses to unconjugated pneumococcal polysaccharide vaccine were observed in 7/8 patients who agreed to be immunized (*Table 8*), two of them showed clinical signs of humoral immunodeficiency and four of CID. Interestingly, none of the patients with specific antibody deficiency reported pneumonia, but all had a history of rhinosinusitis. Other clinical features of the seven subjects with specific antibody deficiency included otitis media (n = 5), warts (n = 3), malignancies (n = 3), severe varicella requiring hospitalization (n = 2) and recurrent herpes simplex virus infections (n = 1). Three out of 43 subjects showed suboptimal antibody levels to tetanus toxoid. For two of them, the time of immunization, if any, was unknown. The remaining patient was re-immunized two years prior to the testing and he also had specific antibody deficiency.

Table 7. Subpopulations of B and T cells in patients with cartilage-hair hypoplasia. Local laboratory or previously published reference values were applied (Boldt, et al. 2014, Wehr, et al. 2008).

Flow cytometry markers	Cell group	Units	N tested	Decreased in N (%)	Normal in N (%)	Increased in N (%)
CD3+	T cells	cells x10^9/l	12	6 (50)	5 (42)	1 (8)
CD4-CD8-	DNT	% of CD3+	11	0 (0)	11 (100)	0 (0)
CD4+CD8+		% of CD3+	11	1 (9)	9 (82)	1 (9)
TCR-α/β+		% of CD3+	11	1 (9)	6 (55)	4 (36)
TCR-γ/δ+		% of CD3+	11	4 (36)	6 (55)	1 (9)
CD4+CD25^{high}CD127^{low}	Regulatory	% of CD3+	11	1 (9)	10 (91)	0 (0)
CD4+		cells x10^9/l	12	6 (50)	6 (50)	0 (0)
CD45RA+CCR7+	Naive CD4+	% of CD4+	11	9 (82)	2 (18)	0 (0)
HLA-DR+CD38-	Activated CD4+	% of CD4+	11	0 (0)	1 (9)	10 (91)
CD45RA-CCR7+	CD4+ TCM	% of CD4+	11	0 (0)	0 (0)	11 (100)
CD45RA-CCR7-	CD4+ TEM	% of CD4+	11	1 (9)	10 (91)	0 (0)
CD45RA+CCR7-	Effector memory CD4+	% of CD4+	11	6 (55)	5 (45)	0 (0)
CD8+		cells x10^9/l	12	10 (84)	1 (8)	1 (8)
CD45RA+CCR7+	Naive CD8+	% of CD8+	11	8 (73)	3 (27)	0 (0)
HLA-DR+CD38-	Activated CD8+	% of CD8+	11	0 (0)	11 (100)	0 (0)
CD45RA-CCR7+	CD8+ TCM	% of CD8+	11	1 (9)	4 (36)	6 (55)
CD45RA-CCR7-	CD8+ TEM	% of CD8+	11	0 (0)	10 (91)	1 (9)
CD45RA+CCR7-	Terminal effector CD8+	% of CD8+	11	0 (0)	3 (27)	8 (73)
CD19+	B cells	cells x10^9/l	12	5 (42)	7 (58)	0 (0)
CD27-IgD+IgM+	Naive	% of CD19+	11	6 (55)	3 (27)	2 (18)
CD21^{low}CD38^{low}	Activated	% of CD19+	11	0 (0)	0 (0)	11 (100)
CD27+IgD+	Marginal zone-like	% of CD19+	11	1 (9)	10 (91)	0 (0)
CD27+IgD-	Switched memory	% of CD19+	11	1 (9)	7 (64)	3 (27)
CD38++IgM+	Transitional	% of CD19+	11	0 (0)	9 (82)	2 (18)
CD38++IgM-	Plasmablasts	% of CD19+	10	3 (30)	5 (50)	2 (20)

DNT double negative T cells, N number patients, TCM central memory T cells, TCR T cell receptor, TEM effector memory T cells.

Table 8. Antibody responses to 10 serotypes of an unconjugated 23-valent pneumococcal polysaccharide vaccine (Pneumovax ®) in eight patients with cartilage-hair hypoplasia. Specific antibody deficiency was defined as a fourfold rise in antibody titers and post-immunization antibody levels ≥ 0.35 μg/ml to less than 70% of serotypes.

Patient	***Age at immunization, years***	***Percentage of reactive serotypes***	***Specific antibody deficiency***
1	23	10%	Yes
2	39	60%	Yes
3	40	20%	Yes
4	44	60%	Yes
5	46	50%	Yes
6	58	30%	Yes
7	65	70%	No
8	67	30%	Yes

We analyzed the laboratory results (as medians) in various age groups (children, young adults 18-44 years and adults >45 years). Patients aged 45 years or older (n = 18) showed higher neutrophil (p = 0.020) and CD4+ (p = 0.025) counts, as well as higher IgA (p = 0.001) and IgG (p = 0.048) levels than younger patients. Children demonstrated higher B cell counts (p = 0.039) and lower concentrations of antibodies to tetanus toxoid (p = 0.043) compared with adult patients.

We did not analyze correlations of B and T cell subpopulations with clinical features due to the modest number of evaluated individuals. The exceptions included CD4+, CD8+, naive thymic T cells and CD19+CD27+ memory B cells that were all tested in the majority of study patients. The type of *RMRP* mutation did not correlate with any clinical or laboratory feature. Multiple regression analysis detected the association between warts and higher IgG levels (p = 0.020), as well as between otitis media and lower neutrophil counts (p = 0.011). No other significant correlations were observed.

5.4. Lung imaging (Study III)

HRCT showed a high prevalence of lung abnormalities in 20 out of 34 patients. Bronchiectasis was diagnosed by HRCT in 10 patients (29%, 10/34), aged from 29 to 68 years. Bronchiectasis was unilateral in two patients and bilateral in eight patients. The most common location of bronchiectasis was in the lower lobes and right middle lobe, but bronchiectasis was observed also in all other lobes. Additional findings on HRCT were acute inflammatory changes (n = 3), fibrosis-like changes (n = 6) and non-specific subpleural nodules of ≤ 0.5 cm in size (n = 8).

MRI showed lung abnormalities in 8 out of 16 patients. However, of the five patients with bronchiectasis for whom both HRCT and MRI were performed, only three patients had MRI score of ≥7 points.

Nevertheless, there was a significant correlation between HRCT and MRI scores. The overall rho was 0.820 ($p < 0.001$) and it ranged from 0.535 to 1.000 for different parameters evaluated, with $p < 0.001$ for most of the variables.

5.5. Malignancies (Study IV)

During the follow-up, 21 out of 80 patients (31%, all adults) developed malignancy, mostly skin cancers and lymphomas (*Table 9*). Importantly, of the 15 patients with non-skin malignancies, more than half (8/15, 53%) demonstrated no clinical manifestations of immunodeficiency prior to the development of cancer.

Table 9. Malignancies diagnosed in 80 patients with cartilage-hair hypoplasia during the 30-year follow-up.

Type of malignancy	*Number of patients*	*Outcome at the end of the follow-up*
Lymphoma	9	Four (44%) alive, five (56%) deceased
Skin cancer	15	Thirteen (87%) alive, one deceased from other type of malignancy and one deceased from causes other than cancer
Basal cell carcinoma	11	
Squamous cell carcinoma	2	
Both types	2	
Lip squamous cell carcinoma	1	Deceased
Myelodysplasia	1	Deceased from causes other than cancer
Neuroendocrine carcinoma	1	Deceased
Plasmacytoma	1	Alive
Thyroid carcinoma	1	Alive
Vocal cord carcinoma	1	Alive

Lymphoma was mostly diagnosed in young adults (median age 32.5 years, range 20.2-45.4 years) and was fatal in 5/9 (56%) of patients (*Table 10*). In one of the survivors, the primary tumor was diagnosed at an early stage (I) due to a scheduled abdominal ultrasound, and the patient remained cancer-free seven years after chemotherapy. Another patient was successfully treated despite the advanced stage (IV) at diagnosis, however, the disease recurred 15 years later. This recurrence was noticed at the scheduled abdominal ultrasound and treated successfully leading to complete resolution. Another survivor had a mucosa-associated lymphoid tissue lymphoma, which was diagnosed at an early stage (I) by gastroscopy performed for abdominal pain and was treated successfully by surgical resection only, with no recurrence during 14 years.

Table 10. Description of lymphoma cases in the cohort of 80 patients with cartilage-hair hypoplasia. Age group (in years) describes age at the diagnosis of lymphoma.

Case	Age group	Type of lymphoma	Location of the primary tumor	Stage at diagnosis	Outcome at the latest follow-up
1	20-30	Diffuse large B cell	Spleen	I	Alive and cancer-free
2	20-30	Nodular sclerosing Hodgkin	Chest	III B	Accidental death
3	20-30	Unspecified	Mediastinum	IV	Death from cancer
4	20-30	MALT	Stomach	I	Alive and cancer-free
5*	30-40	Large cell anaplastic	Lymph node	I A	Two relapses, death from cancer
6	30-40	Diffuse large B cell	Disseminated	IV	Relapse, alive, complete resolution
7	30-40	Small lymphocytic	Lymph nodes	NA	Death from cancer
8	40-50	Burkitt-like	Intra-abdominal adipose tissue	IV	Death from cancer
9	40-50	Diffuse large B cell	Mediastinum	IV	Death from cancer

MALT mucosa-associated lymphoid tissue; NA not available

* This case has been described previously (Taskinen, et al. 2013)

Fifteen patients were diagnosed with skin cancer, mostly basal cell carcinomas. The median age at diagnosis of the first skin cancer was 45.9 years (range 24.5-67.4 years). The median number of skin cancer episodes per patient was two (range 1-9). Altogether, nine patients required therapy for actinic keratosis, and five of them were later diagnosed with skin cancer. All skin malignancies were restricted to the face, head and upper limbs.

SIR for all malignancies in patients with CHH was 9.4 (95% CI 6.4-13) and lymphoid and hematopoietic tissue malignancies (SIR 34, 95% CI 17-60), as well as malignancies of the skin (SIR 20, 95% CI 11-32) were the major contributors to this increased incidence (*Table 11*).

Table 11. The incidence of malignancies in a cohort of 80 patients with cartilage-hair hypoplasia during 1987-2016.

Malignancy	**Obs**	**Exp**	**SIR**	**95% CI**
All malignancies	31	3.31	9.4	6.4-13***
Mouth, pharynx	3	0.06	54	13-140***
Respiratory and intrathoracic organs	2	0.12	15	2.6-48**
Skin	14	0.71	20	11-32***
Endocrine glands	1	0.13	7.8	0.4-34
Ill-defined or unknown	1	0.03	35	2.0-160**
Lymphoid and hematopoietic tissue	10	0.29	34	17-60***

CI confidence interval, Exp expected number of malignancies, Obs observed number of malignancies, SIR standardized incidence ratio
* $p < 0.05$, ** $p<0.01$, *** $p <0.001$

5.6. Mortality (Study IV)

Altogether, 20 out of 80 patients died during follow-up. Causes of death derived from patient records were classified as immunodeficiency-related (n = 15) and -unrelated (n = 5). The former included deaths from infections (n = 4, all from pneumonia), underlying respiratory diseases (n = 4, all patients had bronchiectasis and two also emphysema) and malignancies (n = 7). Deaths from cancer included cases of lymphoma (n = 5), lip squamous cell carcinoma (n = 1) and neuroendocrine carcinoma (n = 1). The median age at death was 40.9 years (24.4 years for death from infections, 40.9 years for death from malignancies and 52.8 years for death from respiratory diseases).

Table 12 describes SMRs for 80 patients with CHH for the period from 1987 to 2016, showing the significantly higher mortality rates (SMR 7.0, 95% CI 4.3-11), mostly due to lymphoma (SMR 60, 95% CI 16-150) and lung disease (SMR 46, 95% CI 9.5-130).

The registered cause of death was “congenital malformations” in four subjects, which referred to CHH itself. However, according to the hospital records, the accurate causes of death in these patients were pneumonia in three and respiratory failure in one. Another patient had “disease of circulatory system” as a registered cause of death, but this subject’s records described death from pneumonia. Therefore, the SMR for infections could not be correctly calculated and the evaluation of SMR for respiratory diseases was also subject to bias.

Table 12. Mortality data in 80 patients with cartilage-hair hypoplasia during 1987-2016.

Age, years	*N*	*Disease mortality*	*Obs*	*Exp*	*SMR*	*95% CI*
0-14	39	All causes	2	0.09	21	2.6-77**
15-29	27	All causes	4	0.48	8.3	2.3-21**
30-44	12	All causes	6	0.79	7.6	2.8-17***
45-59	2	All causes	7	1.03	6.8	2.7-14***
60-74	-	All causes	1	0.45	2.2	0.1-13
Total	80	All causes	20	2.85	7.0	4.3-11***
		All diseases	17	1.93	8.8	5.1-14***
		Neoplasms	7	0.69	10	4.1-21***
		Malignant neoplasms	6	0.68	8.8	3.2-19***
		Lymphoid/hematopoietic neoplasms	4	0.07	60	16-150***
		Malignant neoplasms of lip	1	0.01	75	1.9-420*
		Other malignant neoplasms	1	0.13	7.7	0.2-43
		Other neoplasms	1	0.01	98	2.5-540*
		Circulatory system diseases	2	0.47	4.3	0.5-16
		Respiratory system diseases	3	0.07	46	9.5-130***
		Congenital malformations	4	0.05	76	21-190***
		Alcohol-related diseases	1	0.33	3.0	0.1-17
		Accidents and violence	3	0.90	3.3	0.7-9.8

CI confidence interval, Exp expected and Obs observed number of deaths, N number of patients, SMR standardized mortality ratio
* p <0.05, ** p <0.01, *** p <0.001

5.7. Factors associated with adverse outcome

5.7.1. Factors associated with the development of bronchiectasis (Study III)

Patients with bronchiectasis were significantly older (median age 59.5 years) than patients without bronchiectasis (median age 36.5 years, p 0.023). However, bronchiectasis was diagnosed also in a 29-year-old patient. Patients with bronchiectasis tended to report more chronic cough, sinus infections and pneumonia than patients without bronchiectasis, but the differences were not statistically significant. Six of the study patients reported smoking, four of them had bronchiectasis. However, when controlled for age, the association of smoking with bronchiectasis became insignificant.

Patients with bronchiectasis had higher serum levels of IgG (p = 0.013), as well as higher leukocytes (p = 0.034), lymphocytes (p = 0.008), and NK cells (p = 0.008) compared with patients without bronchiectasis. We also reported a trend for higher counts of T cells, including both CD4+ and CD8+ cells, in patients with bronchiectasis. However, leukocyte, lymphocyte, CD3+, CD4+ and CD8+ T cells, and NK cell counts correlated significantly with age (rho 0.417 (p = 0.014), 0.588 (p = 0.000), 0.484 (p = 0.004), 0.554 (p = 0.001), 0.403 (p = 0.020) and 0.415 (p = 0.016) respectively), while IgG levels correlated with lymphocyte counts (rho 0.439, p = 0.013).

Insufficient levels of serum antibodies to tetanus toxoid were detected in two out of 27 patients, both aged 68 years, and one of them was diagnosed with bronchiectasis. Responses to polysaccharide pneumococcal vaccine were abnormal in those with and without bronchiectasis. Retrospective data on lymphocyte proliferative responses were available for eight patients. Six of them (all without bronchiectasis) demonstrated decreased responses.

Noteworthily, two patients with bronchiectasis had normal results of all the evaluated laboratory parameters, with the exception of low counts of CD27+IgD+ memory B cells.

When we applied multiple logistic regression analysis, of all variables, only higher NK cell counts remained significantly associated with the presence of bronchiectasis (p = 0.026, B coefficient 8.8).

5.7.2. Factors associated with the development of malignancy (Study IV)

Different factors related to the development of certain malignancies. Childhood manifestations associated with lymphoma, while features present in adulthood correlated with skin cancer (*Table 13*). Only recurrent pneumonia in childhood associated significantly with lymphoma (odds ratio 14, 95% CI 1.4-150, p <0.05).

Skin cancer was significantly associated with actinic keratosis and with warts in adulthood, but not in childhood (*Table 13*). All patients with skin malignancies manifested either actinic keratosis (n = 5), warts in adulthood (n = 6) or both (n = 4) prior to the development of skin cancer.

When linear regression analysis was applied, birth length SD score correlated significantly with the age at diagnosis of the first malignancy (p = 0.0029), lymphoma (p = 0.011) and the first skin cancer (p = 0.014), demonstrating that patients with shorter length at birth developed malignancies at an earlier age (*Figure 7*).

Table 13. Risk factors for the development of lymphoma or skin cancer in a cohort of 80 patients with CHH in the multivariate regression analysis adjusted for age and gender.

	Prevalence in patients with outcome, N (%)	**Prevalence in patients without outcome, N (%)**	**OR**	**95% CI**
Development of lymphoma				
Recurrent pneumonia in childhood	3/9 (33)	2/70 (3)	14	1.4-150*
Development of skin cancer				
Actinic keratosis	9/15 (60)	4/65 (6)	20	4.5-88***
Warts in adulthood	10/15 (67)	12/63 (19)	7.6	2.1-27**

CI confidence interval, N number, OR odds ratio
* p <0.05, ** p <0.01, *** p <0.001

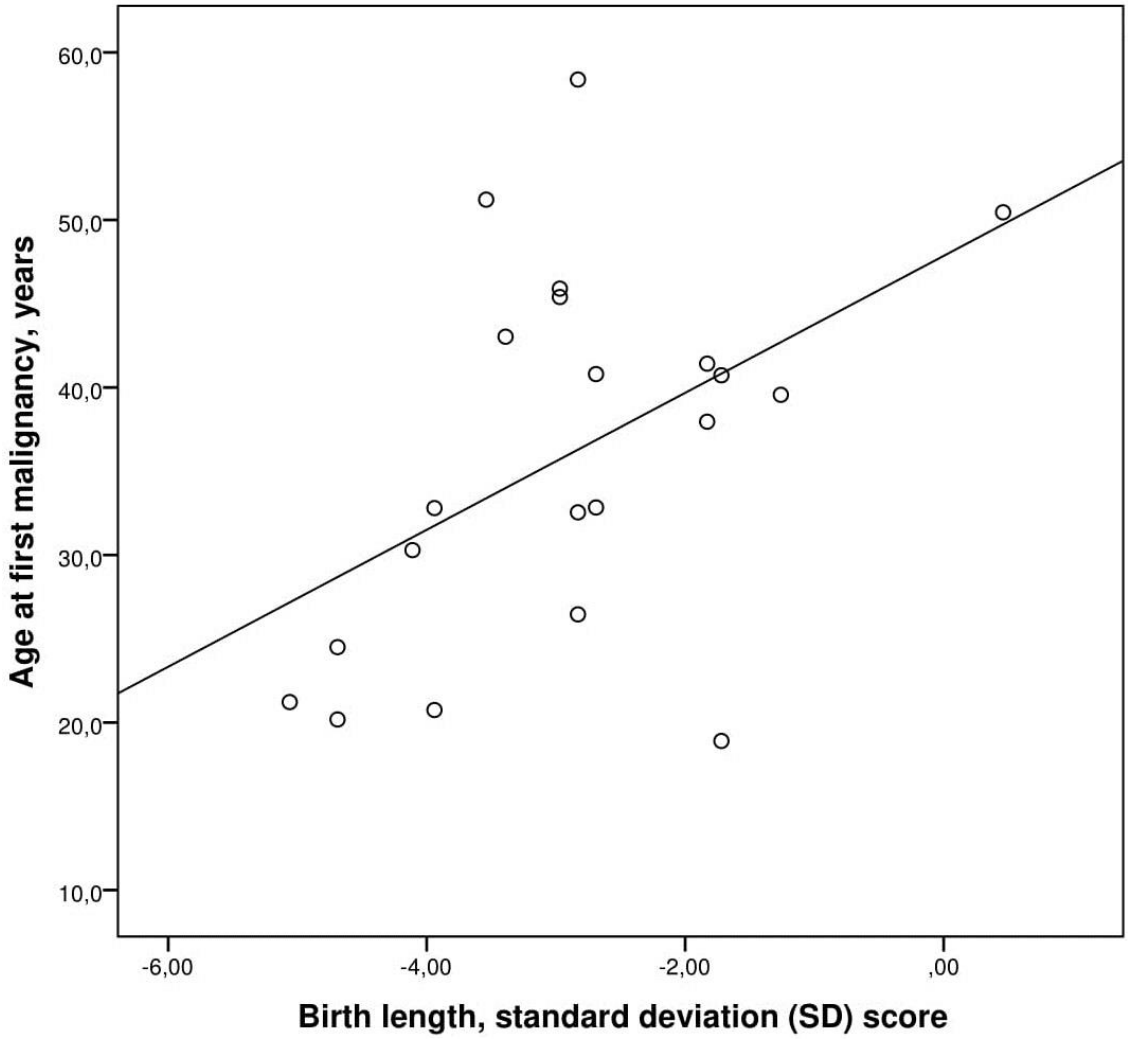
5.7.3. Factors associated with mortality (Study IV)

Due to the small number of infection- and lung disease-related deaths, and the distribution of events in the subgroups of patients, the multivariate analysis could not be performed separately for specific causes of death.

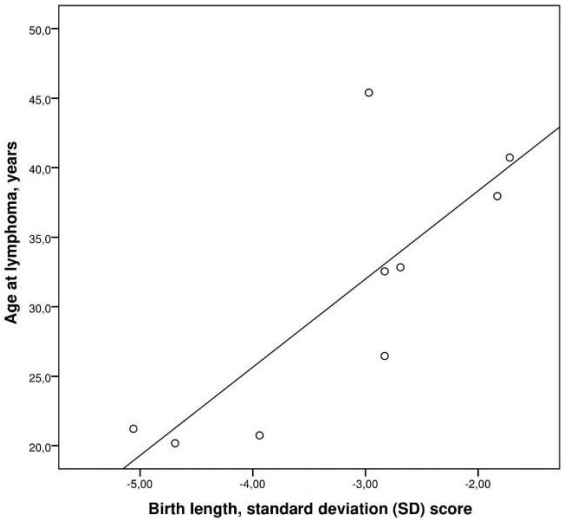
In the analysis of the factors associated with immunodeficiency-related death (due to infections, respiratory disease and malignancies, all combined), Hirschsprung disease, pneumonia in the first year of life, and autoimmune diseases and recurrent pneumonia in adulthood appeared significant in the multivariate regression analysis (*Table 14*).

In addition, severe short stature at birth and symptoms of CID were associated with higher mortality (*Table 15*). Patients with severe short stature at birth (< -4.0 SD) had higher SMR for all causes of deaths than those with normal birth length (> -2.0 SD), (SMR/SMR ratio 5.4, 95% CI 1.5-20) (*Table 15*). Subjects with symptoms of CID had higher all-cause mortality compared with patients with asymptomatic immunodeficiency (SMR/SMR ratio 3.9, 95% CI 1.3-11) (*Table 15*).

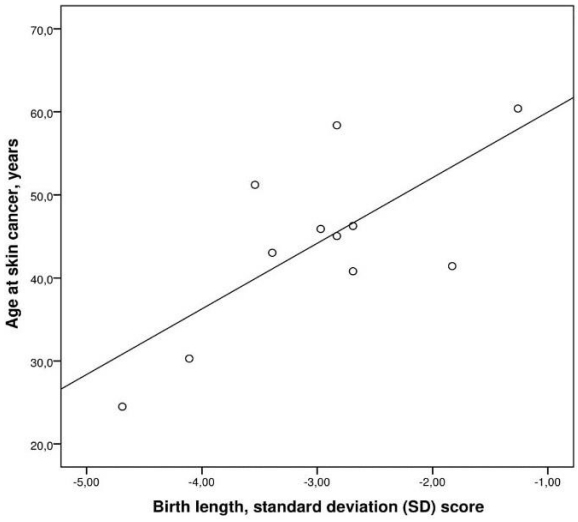
Figure 7. The association of the shorter birth length with the earlier age at development of the first malignancy (A), lymphoma (B) and the first skin cancer (C) in 80 patients with CHH.



A



B



C

Table 14. Risk factors for immunodeficiency-related death in a cohort of 80 patients with cartilage-hair hypoplasia. All study variables were first analyzed by univariate analysis adjusted for age and gender (Model 1). Variables proven significant were then grouped by the time of presentation. Significantly (correlation coefficient >0.6) correlating variables were excluded and the remaining variables were combined for the multivariate analysis, where clinical factors were analyzed separately in childhood and adulthood (Model 2).

Risk factors for immunodeficiency-related death	**Prevalence in patients with outcome, N (%)**	**Prevalence in patients without outcome, N (%)**	**Model 1**		**Spearman's correlation coefficient**		**Included in multivariate analysis**	**Model 2**	
			OR	**95% CI**				**OR**	**95% CI**
Clinical features in the first year of life					0.4: A/B	0.6: B/C			
A. Hirschsprung disease	4/15 (27)	2/65 (3)	15	1.8-130*		0.4: B/D,	+	7.2	1.04-55*
B. Pneumonia	5/14 (36)	4/63 (6	7.8	1.7-36**		B/F, D/K	+	7.6	1.3-43*
Clinical features in childhood					0.7: C/E	0.3: A/F,			
C. Pneumonia	10/15 (67)	13/64 (20)	8.2	2.3-29**	0.3: C/D	D/J	-		
D. Recurrent rhinosinusitis	4/14 (29)	3/61 (5)	6.8	1.1-43*		0.2: A/D,	+	1.6	0.1-21
E. CID	4/15 (27)	3/65 (5)	6.7	1.2-38*		B/I, B/J	-		
Clinical features in adulthood					0.6: F/G,	0.1: A/I,			
F. Autoimmune disease	5/13 (39)	1/65 (2)	42	4.2-410**	F/L, H/I	A/J, B/K,	+	39	3.5-430**
G. CID	7/13 (54)	10/65 (15)	6.8	1.8-26**	0.4: J/K,	D/F, D/I	-		
H. Pneumonia	7/13 (54)	11/65 (17)	5.9	1.6-22**	I/J, I/K	0.0: A/K	-		
I. Recurrent pneumonia	5/13 (39)	2/65 (3)	22	3.3-140**	0.3: F/I		+	19	2.6-140**
J. Recurrent otitis media	4/13 (31)	3/65 (5)	11	1.8-63*	0.1: F/J,		+	4.2	0.4-43
K. Recurrent rhinosinusitis	6/13 (46)	9/64 (14)	5.5	1.4-22*	F/K		+	2.4	0.3-18
L. Low serum levels of IgG in adulthood	4/10 (40)	2/38 (5)	23	2.3-230**			-		

CI confidence interval, CID combined immunodeficiency, Ig immunoglobulin, OR odds ratio

* p <0.05, ** p <0.01

Table 15. Mortality from selected causes in 80 patients with cartilage-hair hypoplasia grouped by birth length SD score and by the category of immunodeficiency (ID).

	Obs	**Exp**	**SMR**	**95% CI**	**Obs**	**Exp**	**SMR**	**95% CI**
Causes of death	**By birth length categories[#]**				**By categories of ID at recruitment**			
	Normal birth length (SD > -2.0, n = 28)				Asymptomatic ID (n = 52)			
All deaths	6	1.47	4.1	1.5-8.9**	10	2.07	4.8	2.3-8.9***
Neoplasms	3	0.44	6.7	1.4-20*	3	0.49	6.2	1.3-18*
Lymphoid/hematopoietic neoplasms	2	0.04	53	6.4-190**	2	0.05	43	5.2-150**
Respiratory diseases	1	0.04	24	0.6-130	2	0.05	44	5.3-160**
	Moderately short birth length (SD -2.0 - -4.0, n = 39)				Symptoms of humoral ID (n = 18)			
All deaths	8	1.11	7.2	3.1-14***	2	0.34	5.9	0.7-21
Neoplasms	3	0.20	15	3.1-44**	0	0.09	0.0	0.0-41
Lymphoid/hematopoietic neoplasms	2	0.02	87	11-320***	0	0.01	0.0	0.0-400
Respiratory diseases	0	0.02	0.0	0.0-190	0	0.01	0.0	0.0-540
	Severely short birth length (SD < -4.0, n = 13)				Symptoms of combined ID (n = 10)			
All deaths	6	0.27	22	8.1-48***	8	0.43	19	8.0-36***
Neoplasms	1	0.05	20	0.5-110	4	0.12	34	9.3-87***
Lymphoid/hematopoietic neoplasms	0	0.01	0.0	0.0-600	2	0.01	180	22-650***
Respiratory diseases	2	0.00	430	52-1600***	1	0.01	79	2.0-440*

CI confidence interval, Exp expected number of deaths, n number of patients, Obs observed number of deaths, SD standard deviation, SMR standardized mortality ratios

* p <0.05, ** p <0.01, *** p <0.001

5.8. Telomere length (Study I)

RTL was measured from 228 samples, including patients (n = 48), first-degree relatives (n = 86) and healthy unrelated controls (n = 94). RTL was not influenced by sex, but we demonstrated a significant negative correlation between RTL and age in mutation carriers (rho -0.482, p < 0.001) and non-carriers (rho -0.498, p < 0.001). Remarkably, the correlation of RTL with age could not be demonstrated in patients with CHH (rho -0.236, p = 0.107).

In the age-group analysis, the vast majority of children with CHH had short telomeres for age (89%, 8/9). Short telomeres were detected in two thirds of patients aged 18.1-40.0 years (11/17, 65%), but in only 27% (6/22) of those aged over 40.1 years (*Table 16*).

Table 16. Relative telomere length in patients with cartilage-hair hypoplasia.

Age group, years	N	RTL, median (range)	Short RTL, N (%)	Average RTL, N (%)	Long RTL, N (%)
6.0-18.0	9	1.12 (0.88-1.31)	8 (89)	1 (11)	0 (0)
18.1-40.0	17	1.08 (0.91-1.72)	11 (65)	2 (12)	4 (23)
40.1-70.8	22	1.06 (0.70-1.81)	6 (27)	8 (36)	8 (37)
All ages	48	1.07 (0.70-1.81)	25 (52)	11 (23)	12 (25)

N number of patients, RTL relative telomere length

5.8.1. RTL in patients, *RMRP* mutation carriers and mutation-negative individuals

Significantly more patients with CHH had short RTL (52%, 25/48) compared with mutation carriers (20%, 15/74, p <0.001) or healthy non-carriers (29%, 31/106, p = 0.011) (*Table 17*). In the comparison of patients with CHH with *RMRP* mutation-negative individuals, significantly more children and young adults with CHH demonstrated short telomeres (p = 0.016 and p = 0.047 respectively), but not older individuals (p = 0.769) (*Table 17*). When RTL itself was compared between patients with CHH and healthy controls, RTL was significantly shorter in children (p = 0.008), but not in adults with CHH (*Figure 8*).

In the control group, we detected age- and sex-matched *RMRP* mutation-negative controls for 40 patients with CHH. In this case-control analysis patients demonstrated significantly shorter RTL (p = 0.017) (*Figure 9*). This difference was explained by shorter RTL in children (p = 0.015, n = 8 pairs), whereas young adults showed only a trend for shorter RTL (p = 0.069, n = 14 pairs) and no difference was seen in older individuals (p = 0.443, n = 18 pairs).

Table 17. Comparison of relative telomere length categories between patients with cartilage-hair hypoplasia, *RMRP* mutation carriers and non-carriers.

Age group, years	N	Short RTL, N (%)	Average RTL, N (%)	Long RTL, N (%)
Patiens				
6.0-18.0	9	8 (89%)	1 (11%)	0 (0%)
18.1-40.0	17	11 (65%)	2 (11.5%)	4 (23.5%)
40.1-70.8	22	6 (27%)	8 (36.5%)	8 (36.5%)
All ages	48	25 (52%)	11 (23%)	12 (25%)
***RMRP* mutation carriers**				
5.0-18.0	17	7 (41%)	6 (35%)	4 (24%)
18.1-40.0	12	3 (25%)	5 (42%)	4 (33%)
40.1-70.8	45	5 (11%)	21 (47%)	19 (42%)
All ages	74	15 (20%)	32 (43%)	27 (37%)
Non-carriers				
6.0-18.0	19	7 (37%)	5 (26%)	7 (37%)
18.1-40.0	40	14 (35%)	17 (43%)	9 (22%)
40.1-70.8	47	11 (23%)	22 (47%)	14 (30%)
All ages	106	31 (29%)	45 (43%)	30 (28%)

N number of patients, RTL relative telomere length

Figure 8. Relative telomere length in patients with cartilage-hair hypoplasia, *RMRP* mutation carriers and non-carriers, grouped by age categories. Children with cartilage-hair hypoplasia demonstrated significantly shorter telomeres (p = 0.008), but not young adults nor adults. o outlier, * extreme outlier.

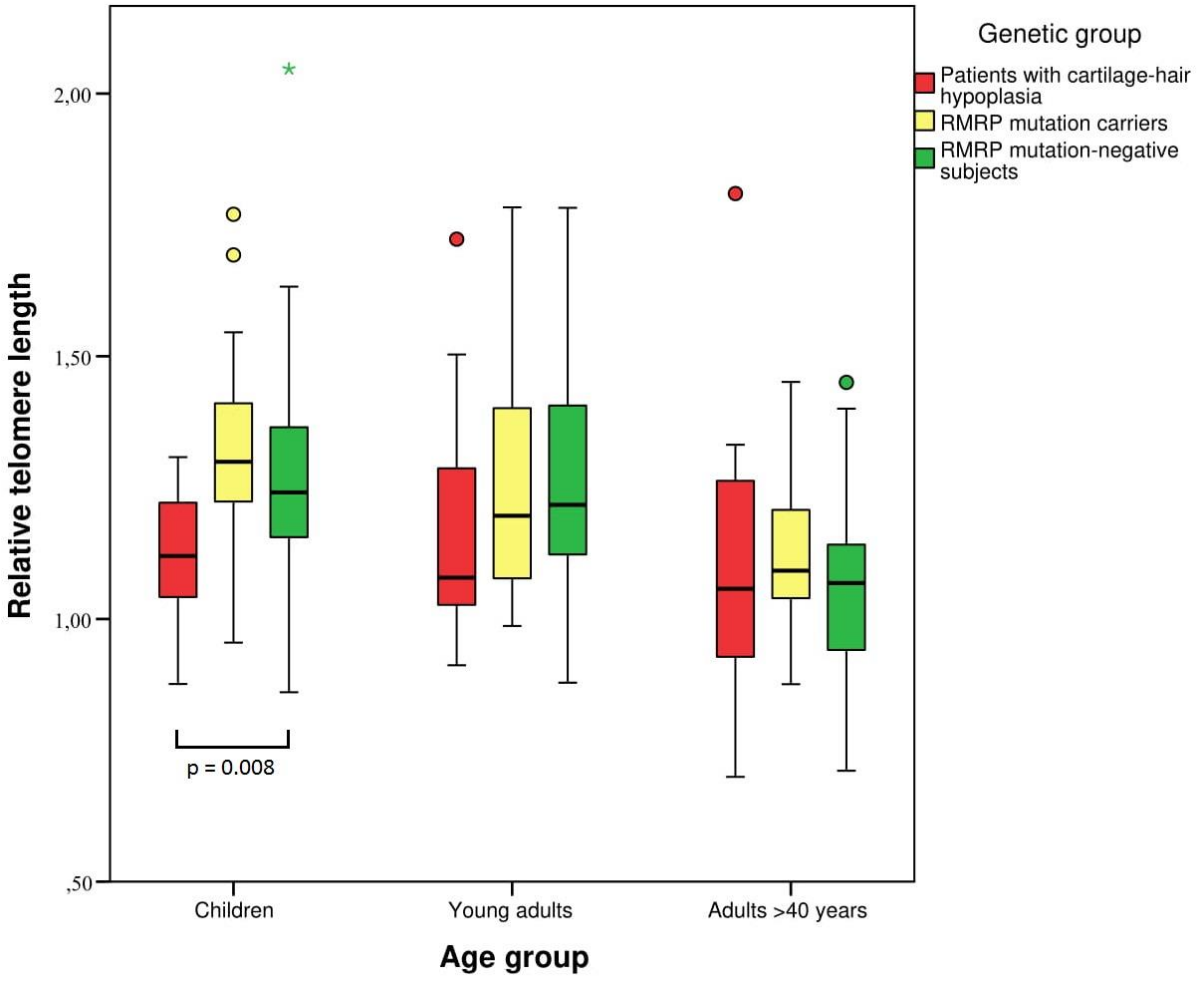
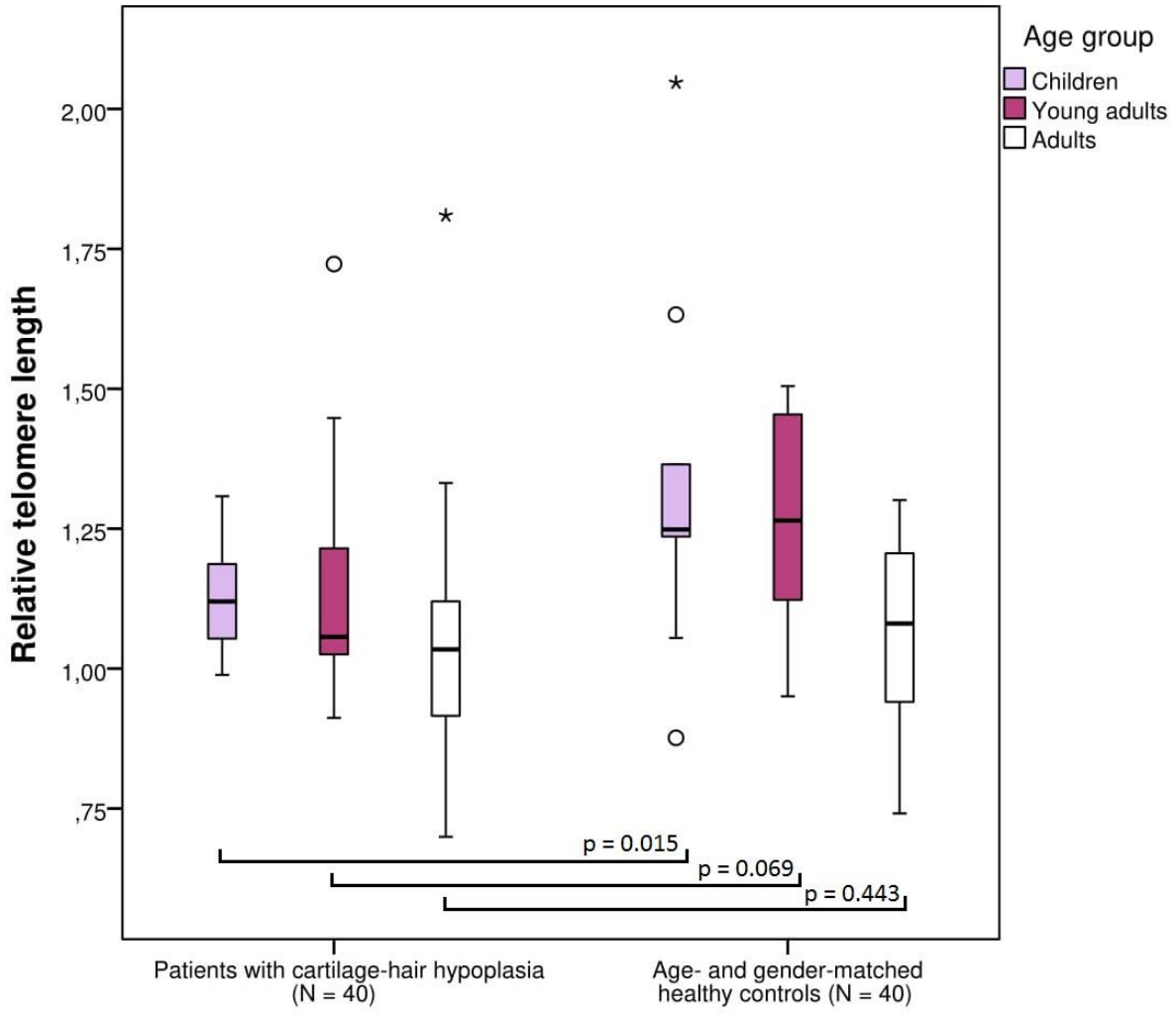


Figure 9. Relative telomere length in 40 patients with cartilage-hair hypoplasia compared with 40 age- and gender-matched healthy *RMRP* mutation non-carriers. Telomere length was significantly shorter in children with CHH (p = 0.015, n = 8 pairs), but not young adults (p = 0.069, n = 14 pairs) nor adults (p = 0.443, n = 18 pairs). o outlier, * extreme outlier.



5.8.2. Correlation of RTL with CHH-related features

We examined all available clinical and laboratory data to find correlations with RTL itself or RTL categories. Variables included sex, the type of *RMRP* mutation, the degree of growth failure, the history of obesity, smoking, blood transfusions, immunoglobulin substitution, hormone or immunosuppressive therapy, various infectious manifestations separately or in combinations, fibrosis-like lung changes, malignancies, and analyzed laboratory parameters. No significant correlations were detected.

6. DISCUSSION

This study explored clinical and pathogenetic features and the associated outcomes in a large cohort of Finnish patients with CHH. Previous research has benefited from an exceptionally large number of patients with CHH living in Finland. These landmark studies substantially expanded our knowledge of CHH and led to the discovery of the genetic defect underlying this disease. Our study described numerous novel findings adding to our understanding of the pathogenesis, immune phenotype, disease course and prognosis in CHH.

6.1. Clinical and immunologic phenotype

We analyzed clinical and immunologic data in the unique sample of 56 pediatric and adult subjects with CHH (Study II). We could not demonstrate any significant correlations between clinical features and laboratory parameters, and few patients reported serious complications despite abnormal laboratory values. Consistently, in Study IV we showed that clinical symptoms were more valuable as prognostic factors than laboratory parameters.

Our long-term follow-up Study IV allowed for determination of clinical patterns of immunodeficiency in CHH. Half of the patients in our cohort remained clinically asymptomatic for decades. Some *RMRP* gene mutations induce a more severe immunodeficiency (Thiel and Rauch 2011), and the predominance of n.71A>G mutation in Finnish patients can result in milder phenotypes. However, many of the asymptomatic subjects either progressed to the late-onset immunodeficiency in adulthood, or developed malignancy, often fatal. Therefore, all patients with CHH, even completely asymptomatic, should be carefully followed.

All of our patients in Study IV who had symptomatic immunodeficiency presented with infections typical for defects in humoral immunity, including recurrent RTI, bacterial skin infections and/or sepsis. The spectrum of pathogens causing these infections was also consistent with humoral immunodeficiency. This clinical presentation is in contrast with low rates of hypogammaglobulinemia in our cohort. However, many patients had low counts of B cells. Altogether, these findings suggest that despite having normal or even high levels of IgG, subjects with CHH behave like antibody-deficient patients and are unable to combat common bacterial pathogens. This pattern should be recognized, and patients suffering from recurrent infections should be considered for prophylactic antibiotics and/or a trial of IGRT irrespective of serum IgG levels.

Despite low counts of B cells in many subjects, IgG levels were normal in all but one patient in Study II, including measurements available prior to the commencement of IGRT in two subjects. Paradoxically, high IgG levels were the most common immunoglobulin abnormality in patients from Study IV. Serum immunoglobulin levels are maintained by long-lived plasma cells, and thus do not reflect the ongoing effective immunoglobulin production (Blanco, et al. 2018). In patients with classical clinical signs of humoral immunodeficiency, including some

patients with CHH, IGRT should be trialed irrespectively of IgG levels. This approach has shown efficacy in preventing infections in patients with hyper-IgE syndrome induced by *STAT3* mutations, who benefit significantly from IGRT despite having normal levels of IgG (Chandesris, et al. 2012). The potential efficacy of IGRT is supported by the finding of a large proportion of patients with CHH presenting with low memory B cell counts. The observed pattern of abnormalities in B cell subpopulations is consistent with impaired B cell production and germinal center defect, while decreased counts of CD27+ B cells suggest maturation or survival defect (Driessen, et al. 2011), supporting the consideration of IGRT in CHH.

The inclusion of children and elderly subjects into the study allowed us to record and analyze aged-related trends in immunological parameters. In general population, serum immunoglobulin levels increase with age, whereas total lymphocyte and B cell counts reach the nadir at around six months of life and slowly decline thereafter (Blanco, et al. 2018). We observed the same changes in the laboratory parameters in our Study II cohort. However, higher numbers of CD4+ cells in adults with CHH and the increased CD8+ and CD16/56+ cell counts in children with CHH remain to be explained in future studies.

Protective levels of anti-tetanus toxoid antibodies were present in most of the subjects in Study II. By contrast, we reported for the first time a high prevalence of specific antibody deficiency in patients with CHH (88%). Of CHH individuals with specific antibody deficiency, 57% had clinical features suggesting CID. However, in Study IV, in which we identified 12 more patients who had been tested for the pneumococcal polysaccharide vaccine responses (abnormal in seven), specific antibody deficiency showed no correlation with clinical symptoms or outcomes.

In contract to the previous studies in Finnish patients with CHH, we demonstrated high rates (20%) of opportunistic infections (Study IV). These were, however, mostly confined to mucocutaneous viral and fungal infections. The prevalence of cutaneous warts in patients with CHH was higher than in healthy population (33-34% vs 5%) (Leiding and Holland 2012). At the time of the follow-up visit, six children and eight adults presented with warts. Interestingly, as many as 50% of the subjects with specific antibody deficiency reported a history of warts in Study II, suggesting more clinically evident CID.

Basal cell carcinoma was the most common malignancy in our patients, however, it is rarely described in individuals with other PID. Single cases have been reported in patients with common variable immunodeficiency (Todorovic, et al. 2014) and WHIM syndrome (Beaussant Cohen, et al. 2012). The latter study highlights the role of human papilloma virus in the development of skin cancer, which is also demonstrated by the increased risk of cutaneous malignancy in epidermodysplasia verruciformis (Accardi and Gheit 2014). We showed that warts in adulthood, but not in childhood, were associated with the development of skin cancer in CHH. This suggests the benign nature of warts in children, but in adulthood warts can indicate impaired skin immunity and can result in malignant transformation.

Consistent with previous studies, we reported a high incidence of malignancy in CHH, mostly due to lymphomas and skin cancers (Study IV). Compared with earlier reports (Taskinen, et al. 2008), we described improved survival after the diagnosis of lymphoma (4/9 patients alive, 44%), mostly due to early detection of tumors. Our data underscores the importance of regular cancer screening and thorough diagnostic work-up of symptomatic patients.

6.2. The role of telomere machinery in CHH

We expanded the knowledge of CHH pathogenesis by demonstrating shorter telomeres in children with CHH (Study I). Our results have been later confirmed by Aubert et al (Aubert, et al. 2017). They measured telomere length and telomerase activity in lymphocytes from 15 patients with CHH (including two adults) and eight *RMRP* mutation carriers. They used a different method of telomere measuring (flow-fluorescent *in situ* hybridization) and compared their results with a much larger cohort of healthy individuals (n = 835). Consistent with our findings, telomeres were significantly shorter in subjects with CHH, and impaired telomerase activity was also described. Our discovery of shorter telomeres in children, but not adults with CHH, was later supported by the demonstration of the same trend in patients with pathogenic variants in telomere machinery genes (Alder, et al. 2018).

Disorder of telomere maintenance add to the impaired cell biology and together can explain the majority of CHH features, including short stature, immunodeficiency, increased risk of malignancy, hypoplastic anemia, lung disease, enteropathy and impaired spermatogenesis (Alder, et al. 2018, Wagner, et al. 2018). T cells in CHH demonstrate increased apoptosis (de la Fuente, et al. 2011), and the underlying mechanism for this can involve the activation of DNA damage response triggered by critical shortening of telomeres (Alder, et al. 2018). Very recently, T cell immunodeficiency has been demonstrated in a cohort of patients with telomeropathies in the absence of bone marrow failure (Wagner, et al. 2018). Interestingly, the most common infections in this cohort were caused by herpes viruses, echoing the predisposition to severe varicella in CHH. Moreover, abnormalities in T cell populations in subjects with short telomere syndromes included depletion of naive T cells and accumulation of effector memory CD8+ cells, similar to our findings in patients with CHH in Study II.

The pathogenesis of malignancies in CHH is probably multifactorial and includes the defects in cell proliferation and apoptosis. Disorders of telomere maintenance, such as dyskeratosis congenita, are characterized by increased susceptibility to malignancies, mostly head and neck squamous cell carcinomas and skin cancers (Alter, et al. 2009). Our findings of impaired telomere biology offer an additional risk factor for the increased cancer rates in patients with CHH. In Study IV, we demonstrated that actinic keratosis was the most significant risk factor for skin cancer, which developed on sun-exposed skin areas in patients with CHH. Ultraviolet exposure can exhibit detrimental effect on cells with impaired telomere and DNA repair machinery, explaining the high susceptibility to skin malignancies in CHH (Anic, et al. 2013).

Lung fibrosis is an established manifestation of dyskeratosis congenita, and short telomeres have been demonstrated also in cases of idiopathic pulmonary fibrosis and early-onset emphysema (Alder, et al. 2018, Armanios, et al. 2007). Apart from bronchiectasis, we reported high rates (18%) of fibrosis-like changes in subjects with CHH in Study III and we also described two patients with fatal emphysema in Study IV. Lung disease in patients with telomeropathies manifests in adulthood (Alder, et al. 2018), and in Study IV we demonstrated that, compared with deaths due to infections and malignancies, mortality attributed to respiratory disease in patients with CHH was highest in the older age group. Therefore, some cases of lung disease in CHH can be linked to telomere abnormalities and this hypothesis requires further studies.

We acknowledge that, although telomere length differed significantly in patients with CHH and healthy controls, the actual numbers behind these differences were rather small, and additional research is necessary to confirm these findings and investigate whether short telomeres underlie clinical features of CHH.

6.3. Lung disease in CHH

We reported a high prevalence (29%) of bronchiectasis in a random cohort of 34 Finnish patients with CHH (Study III). This result corroborates previous studies of moderately or severely affected CHH cohorts, where the prevalence ranged from 43 to 52% (Bordon, et al. 2010, Toiviainen-Salo, et al. 2008). We showed that bronchiectasis can develop in patients with CHH even in the absence of apparent immunodeficiency. Most of the patients in Study III had no or minor respiratory symptoms and had never underwent lung HRCT previously, in line with the lack of clinical indication for detailed lung imaging. However, half of the subjects with bronchiectasis reported daily cough ongoing for at least one year. This calls for regular lung imaging even in mildly symptomatic patients.

Clinical significance of asymptomatic bronchiectasis in healthy persons is an area of debate. However, in patients with immunodeficiency, pulmonary diseases contribute to poor prognosis (Sullivan KE 2014). In patients with CHH, lung changes should thus be actively searched for and treated if present. In Study III, we diagnosed bronchiectasis in a patient as young as 29 years and, in Study IV, we described additional cases of bronchiectasis in 10-, 14- and 16-year-old children. This emphasizes the necessity of early screening, in order to commence proper investigations and treatment in a timely manner.

The high prevalence of bronchiectasis in our cohort could be partially explained by female gender predominance, as bronchiectasis occur more commonly in women than men (Kwak, et al. 2010). However, of the 10 patients with bronchiectasis in Study III, three were men, and the proportion of women was actually higher in the group of patients without bronchiectasis. We identified additional 11 patients diagnosed with bronchiectasis in Study IV, all females. However, when all 37 cases of bronchiectasis from Study IV were analyzed, the proportion of

females in patients with and without bronchiectasis did not differ significantly (16/21 and 13/26 respectively, p = 1.000).

The prevalence of bronchiectasis in the general population increases with age (Kwak, et al. 2010), and in our sample, six out of 10 patients with bronchiectasis were aged over 58 years. The prevalence of bronchiectasis in this age group in general population is as high as 15% (Kwak, et al. 2010), but it was significantly higher (55%) in our patients. Furthermore, the majority (9/11) of the additional cases of bronchiectasis from Study IV were diagnosed in subjects younger than 58 years.

The history of sinus infections correlates positively with the presence of bronchiectasis in the general population (Guilemany, et al. 2009). However, we could not confirm this correlation in Study III. Still, there was a trend for more sinus and middle ear infections, as well as pneumonia in CHH patients with bronchiectasis. Our results could be affected by the retrospective nature of clinical data and by the small number of patients included in the study.

We previously reported higher numbers of T cells and higher IgG levels in CHH patients with increased susceptibility to infections (Makitie, et al. 1998, Makitie, et al. 2000). In Study III, we showed the same trend in CHH patients with bronchiectasis. The possible explanations include smoking, chronic infections or inflammation in the respiratory tract and bronchiectasis-induced systemic immune response (Daheshia, et al. 2012). We recognize the possibility of bias arising from exclusion of three subjects receiving IGRT from the analysis of IgG levels. However, when we included pre-IGRT IgG levels available for two patients into the analysis, the results were equal and became even more significant. Whatever the underlying mechanisms are, clinically those with higher, rather than lower, cell counts and IgG concentrations surprisingly appeared to be at risk of having bronchiectasis. However, these laboratory parameters correlated with age and may just represent cumulation of bronchiectasis in the older age group in Study III.

Almost one third of patients had previously been diagnosed with asthma by a physician, but, unfortunately, we were not able to assess the individual diagnostic criteria for asthma. Given that the prevalence of asthma in the general population of Finnish adults is around 6% (Kotaniemi, et al. 2002), this diagnosis was significantly more common in patients with CHH (21-24%). Asthma may have been misdiagnosed as the cause of respiratory symptoms in some of the patients. We have later demonstrated the high incidence of autoimmunity in patients with CHH, and asthma diagnosis may thus reflect immune dysregulation (Vakkilainen, et al. 2018). The latter can also explain the higher prevalence of anamnestic allergic rhinitis in our cohort (41-44%) when compared with the general Finnish population (15-25%) (Remes, et al. 1998). On the other hand, chronic upper respiratory symptoms triggered by infections can be misinterpreted by patients or misdiagnosed by physicians as allergy. Asthma evaluation by spirometry in CHH is complicated due to short-stature since the height-related values may be normal even when pulmonary function is diminished, whereas age-related values may be falsely low. Incorporating sitting height measurement into spirometry assessment of patients with CHH may be beneficial. We did not assess lung diffusion capacity and it should be evaluated in future studies.

Chronic respiratory symptoms in patients with CHH should not be attributed to asthma until lung imaging is performed. Correct diagnosis is crucial for management, for instance, inhaled corticosteroids in adults with bronchiectasis increase the risk of lung infections, and thus should be used only after obtaining objective evidence of their benefit (Chang, et al. 2018).

The comparison of HRCT and MRI scores in our study patients confirmed that HRCT detects minor pathological changes in small airways better than MRI. Lung HRCT remains the gold standard for diagnosing bronchiectasis, but we demonstrated a good performance of lung MRI in evaluation of bronchiectasis in patients with CHH. Lung MRI may have a role in the follow-up of lung status, reducing repeated radiation exposure.

6.4. Factors associated with adverse outcomes

There are several obstacles in investigating prognostic correlations in CHH cohorts. First, the definition of increased susceptibility to infections is not universal and varies among studies (*Table 2*). Second, children with CHH may be mildly symptomatic and develop clinical signs of immunodeficiency only in adulthood (Horn, et al. 2010), and, therefore, studies performed in pediatric population can be biased. In addition, laboratory immunologic parameters can fluctuate with time, challenging the correlation of phenotype with a single laboratory measurement. In addition, only prospective studies with prolonged follow-up can shed light on the prognostic factors present in childhood for mortality or the development of malignancies in adulthood. Finally, the rarity of the disease results in small number of patients recruited to the studies, complicating the statistical analysis.

We therefore, conducted a prospective long-term follow-up study in a large cohort of 80 patients with CHH. This landmark study (IV) provided multiple risk factors for immunodeficiency-related mortality and for the development of malignancies.

Hirschsprung disease is a well-recognized co-morbidity in patients with CHH. It demonstrates a more severe clinical course and poor prognosis in CHH (Makitie, et al. 2002, Makitie, et al. 2001). Our data confirmed this association and linked Hirschsprung disease with mortality in subjects with CHH. Two mechanisms can explain these findings. First, the high prevalence of Hirschsprung disease in individuals with CHH suggests that RMRP contributes to the pathogenesis of Hirschsprung disease, probably via impaired gene regulation. Cases of CHH with Hirschsprung disease may thus represent severely abnormal RMRP function resulting in a more profound immunodeficiency. Second, enterocolitis associated with Hirschsprung disease predisposes patients to infections with gut microorganisms and interferes with gastrointestinal mucosal immunity (Gosain and Brinkman 2015, Zhao, et al. 2010).

In addition, shorter birth length correlates with increased risk of infections in CHH (Makitie and Kaitila 1993, Rider, et al. 2009), and we demonstrated that birth length Z score < -4.0 associated with mortality. Patients with CHH and less severe skeletal manifestations can also

develop serious lung damage or fatal malignancies (Klemetti, et al. 2017), however, extreme short stature probably reflects the higher degree of the disruption of RMRP function and can thus associate with more profound immunodeficiency.

Recently, the development of autoimmune conditions has been shown to correlate with increased mortality in patients with CHH (Vakkilainen, et al. 2018). Using a different approach, we confirmed these findings in Study IV, which included 80 out of 104 patients from the previous report and showed that autoimmunity is a risk factor for immunodeficiency-related death.

Importantly, many risk factors for adverse outcomes in CHH can be identified early in childhood. These include Hirschsprung disease, birth length score less than -4.0 SD, pneumonia in the first year of life and symptoms of combined immunodeficiency. These features can predict a more severe disease course and should prompt consideration of aggressive treatment strategies, including HSCT.

After the introduction of newborn screening for SCID using T cell receptor excision circles, some patients with CHH can present for the evaluation and consideration of HSCT. In asymptomatic cases, if clinicians and caregivers opt for watchful waiting, patients should be closely followed-up to identify specific features associated with adverse outcome and necessity of HSCT should be re-evaluated as needed.

We could not identify risk factors for the development of fatal or non-fatal malignancy in our cohort when patients with all registered malignancies were included into analysis. This suggests that skin cancer and lymphoma arise from different mechanisms in CHH. In addition, some of the malignancies reported in our patients (for example, thyroid carcinoma) are not associated with PID.

When cases of lymphoma were analyzed, recurrent pneumonia in childhood was identified as the only risk factor. However, we also described patients with CHH and clinically asymptomatic immunodeficiency who developed malignancies. Hence, while profound immunodeficiency, illustrated by recurrent RTI, associates with lymphoma, other mechanisms underlie the development of malignancies in mildly symptomatic patients. Further studies should identify these pathways on the molecular and cellular levels.

6.5. Implications for management

Our demonstration of shorter telomeres in patients with CHH provides several potential therapeutic implications. Severe hypoplastic anemia in CHH can be life-threatening and can require HSCT (Taskinen, et al. 2013, Williams, et al. 2005). The pathogenesis of bone marrow failure in CHH can involve impaired telomere machinery and therapeutic strategies can be adopted from other telomeropathies. Androgens have been used successfully to treat anemia in patients with dyskeratosis congenita (Calado and Cle 2017), and their efficacy in bone

marrow failure in CHH should be investigated. Subjects with short telomere syndromes are radiation-sensitive and prone to develop treatment-related toxicity with conventional HSCT regimens and other T cell cytotoxic drugs (Wagner, et al. 2018). Therefore, our findings should be explored further in order to evaluate the necessity of the adjustment of therapeutic regimens in patients with CHH.

Infections and malignancies are the most common causes of death in patients with PID (Mortaz, et al. 2016) and this holds true also for those with CHH. Management decisions should thus focus on prevention and proper treatment of infections, as well as careful follow-up for early detection of malignancies. In addition, we demonstrated for the first time, that lung diseases contribute significantly to the mortality of patients with CHH. Therefore, aggressive treatment approaches should be implicated, and patients' management should include the prevention of RTI, timely detection of bronchiectasis and consideration of HSCT in cases of lung disease unresponsive to other therapies.

Pulmonary management in patients with CHH should aim at prevention of lung infections and bronchiectasis. This should include proper immunization against pathogens causing respiratory infections, including pneumococcal and annual influenza vaccines. Patients should be advised against smoking and, in case of proven respiratory allergies, should avoid aeroallergens. In addition, addressing co-morbidities influencing pulmonary status, such as chronic rhinosinusitis, obesity, skeletal deformities, gastroesophageal reflux and vitamin D deficiency, is important to prevent lung morbidities (Chang, et al. 2018).

The treatment of the existing bronchiectasis focuses on hindering further lung damage and on proper treatment of exacerbations. Pulmonary rehabilitation and exercise have demonstrated efficacy in adults with bronchiectasis (Chang, et al. 2018). Antibiotics, including macrolides, are the cornerstone of therapy and prevention of flares in patients with bronchiectasis irrespective of underlying pathology (Chang, et al. 2018). Prolonged treatment with clarithromycin has been successful in CHH children with lung disease, resulting in the improvement of bronchiectasis in one of the patients (Bailly-Botuha, et al. 2008).

While IGRT is of undoubted benefit in patients with symptomatic hypogammaglobulinemia, the commencement of this therapy is questionable in those with normal IgG levels, including the majority of patients with CHH (*Table 2*). Only several case reports describe the clinical course of CHH patients after initiation of IGRT for recurrent RTI (Ammann, et al. 2004, Horn, et al. 2010, Moshous, et al. 2011). Of the five subjects (four with hypogammaglobulinemia), only two benefited from therapy, other three continued to experience recurrent infections, including one with a fatal outcome. However, suboptimal immunoglobulin dosing may have affected poor clinical response described in these studies. For example, in a 4-year-old boy, the dose of 0.4 g/kg of intravenous immunoglobulin was used by Ammann at al, which resulted in trough levels of IgG between 5.1 and 6.0 g/l (Ammann, et al. 2004).

Occasionally, IGRT and prophylactic antibiotics induce regression and even resolution of bronchiectasis in primary antibody deficiencies, but this cannot be shown consistently (Baris, et al. 2011, Gregersen, et al. 2010). IGRT reduces the rates of pulmonary infections in patients with humoral immunodeficiency but does not prevent the decline in lung function (Baumann, et al. 2018). Our findings of higher IgG levels in patients with bronchiectasis suggest that management decisions have to be made on individual basis and cannot be based on low IgG levels alone. However, IGRT can be insufficient to prevent lung damage and death from end-stage lung disease in patients with CHH, and HSCT should be considered in severe cases. Importantly, the progression of bronchiectasis in patients with CHH can be halted by HSCT (Bordon, et al. 2010).

The outcome of patients with CHH after HSCT has been reported in a series of 3, 6, 13 and 16 patients with survival rates of 100%, 100%, 83% and 63% respectively (Bordon, et al. 2010, Guggenheim, et al. 2006, Ip, et al. 2015, Kavadas, et al. 2008). Mortality after HSCT was attributed mostly to infections, including disseminated adenovirus, cerebral mucormycosis, pneumococcal sepsis and pseudomonal pneumonia (Bordon, et al. 2010, Ip, et al. 2015). The majority of surviving patients achieved full reconstitution of B and T cell numbers and function (Guggenheim, et al. 2006, Ip, et al. 2015) and the quality of life was improved (Bordon, et al. 2010, Ip, et al. 2015). As in other PID, early HSCT can be life-saving in CHH, while HSCT performed after the onset of opportunistic infections can be fatal (Moshous, et al. 2011).

HSCT in patients with PID decreases the risk of malignancies (Kamani, et al. 2011). No cancer cases have yet been reported in patients with CHH who had received HSCT and had been followed-up for up to 22 years (Bordon, et al. 2010, Guggenheim, et al. 2006, Kavadas, et al. 2008). Therefore, HSCT offers a preventive approach if prognostic indicators could select candidates for HSCT before the development of malignancies.

With the advent of newborn screening for SCID, some CHH patients with low numbers of T cell receptor excision circles, reflecting poor T cell production, will be diagnosed very early in life. The optimal management of such cases remains debatable. Early HSCT may provide cure for the underlying immunodeficiency before the potential onset of severe infections. However, given the mild clinical course of some patients with CHH and good survival after HSCT in adults with PID (Fox, et al. 2018), a more conservative approach could be possible, with careful observation and timely detection of disease progression. Therefore, implication of the knowledge of risk factors for the development of severe complications is crucial to detect subjects most likely to benefit from HSCT.

Traditionally, HSCT has been rarely used in Finnish patients with CHH, and no HSCT has been performed in our study subjects. However, HSCT has been considered for some of these patients late in the disease course, but, unfortunately, was declined by clinicians due to the poor health condition of the subjects. Therefore, HSCT should be considered early for selected patients with multiple risk factors for adverse outcome detected in our studies.

The possible clinical implications of our studies in the management of patients with CHH are presented in *Table 18*.

Table 18. Implications in the management of Finnish patients with cartilage-hair hypoplasia.

Research findings	**Implications for management**
Certain features associate with morbidity and mortality in patients with CHH. Patients with CID show the most severe clinical course.	Assess risk factors for early mortality to choose appropriate management strategies, for example, consider HSCT in patients with CHH and CID.
Hirschsprung disease associates with immunodeficiency-related mortality in CHH.	Acknowledge that Hirschsprung disease is one of the markers of severe disease course in CHH.
Birth length score less than -4.0 standard deviation associates with mortality. Patients with shorter birth length develop malignancies at an earlier age.	Acknowledge that shorter birth length carries poor prognosis in CHH.
In addition to CID and Hirschsprung disease, pneumonia in the first year of life, and recurrent pneumonia and autoimmunity in adulthood associate with immunodeficiency-related death in CHH.	Acknowledge that several other risk factors associate with adverse outcomes in CHH.
Infections and respiratory diseases are among the most common causes of death in patients with CHH.	Consider aggressive management (prophylactic antibiotics, IGRT, HSCT) in patients with recurrent RTI and pulmonary complications.
The majority of patients with CHH, even severely symptomatic, present with normal or elevated serum IgG levels. The most common pattern and etiology of infections are similar to infections in antibody-deficient patients. Patients show high prevalence of bronchiectasis and die of infections and lung disease. Many patients demonstrate specific antibody deficiency and/or decreased counts of B cells, including low memory B cells.	Consider antibiotic prophylaxis and/or IGRT based on clinical manifestations, irrespectively of serum IgG levels.
Patients can develop adult-onset immunodeficiency or malignancy that can be fatal.	Follow-up all patients, even completely asymptomatic.
Lung changes, mostly bronchiectasis, are highly prevalent in CHH, and respiratory symptoms should not be attributed to asthma until other underlying causes are excluded. Lung MRI findings correlate well with HRCT results and decrease radiation exposure.	Perform lung imaging by HRCT and/or MRI in patients with chronic respiratory symptoms.
Patients have increased incidence of malignancies, mostly lymphoma and skin cancer. Early detection of lymphoma can result in successful therapy with complete resolution.	Screen for cancer regularly (clinical examination, abdominal ultrasound).
Skin cancer is common in CHH and is limited to sun-exposed skin areas. Actinic keratosis is the strongest risk factor for skin malignancy in patients with CHH.	Educate patients about the importance of sun protection to reduce the risk of skin cancer.
Pulmonary complications are highly prevalent in CHH.	Advise patients against smoking.

CHH cartilage-hair hypoplasia, CID combined immunodeficiency, HRCT high-resolution computed tomography, Ig immunoglobulin, IGRT immunoglobulin replacement therapy, MRI magnetic resonance imaging, RTI respiratory tract infections.

7. STRENGTHS AND LIMITATIONS

The major strength of our research is the novelty of the reported data. Our studies were first to describe the prevalence of bronchiectasis in the general CHH population, the detailed data on T and B cell subpopulations and vaccine responses, telomere length, and, most importantly, long-term follow-up data and risk factors for mortality in patients with CHH.

Other advantages of our studies include large numbers of participating patients, allowing for adequate sample size for statistical analysis. The inclusion of adult patients provided novel valuable information on the disease course in CHH patients surviving into adulthood. The accuracy of data collection was maximized in Study IV by using all available information from all Finnish health registries, as well as by thorough analysis of all patient records.

The major limitation of studies I-III is the retrospective nature of clinical data. We overcame this in Study IV which produced prospective and long-term follow-up health data.

Further limitations include possible selection bias, as we recruited patients regardless of their clinical presentation. One can speculate that the patients who decided to take part in the study may have experienced more symptoms and agreed to participate in order to receive medical evaluation and treatment. However, most of the patients in our cohort reported no current complaints during visits.

Another limitation of our studies I-III is the use of a single laboratory measurement per patient. Results of immunologic tests in patients with CHH fluctuate and predicting clinical course based on cross-sectional laboratory evaluation may be challenging. The participation of patients from all age groups could influence the analysis as the symptoms may cumulate and the results of laboratory tests may differ in older patients. The latter was overcome by age-appropriate reference values and multiple regression analysis.

In Study I, the small number of healthy controls may increase the risk of bias. Another limitation is the use of RTL, which did not allow for the estimation of telomere length distributions and for detection of critically short individual telomere ends. Measurement of telomeres from peripheral blood with PCR has high variability, in part due to fluctuations of telomere length in leukocytes (Alder, et al. 2018). However, our results have been confirmed in later study in which a different measurement method was applied (Aubert, et al. 2017).

We acknowledge the limitations of our definitions of humoral or combined immunodeficiencies based on the reported symptoms only, but we feel that, given the diversity and fluctuation of laboratory results in patients with CHH, this may have some practical implications. In Study IV, patients categorized as having CID showed higher mortality. This suggests that our clinical categorization of immunodeficiency correlates well with prognosis of the patients and can be adopted in clinical practice.

8. FUTURE PROSPECTS

Our results provide several new research directions. Lung disease contributes significantly to the morbidity and mortality of patients with CHH. Further studies should address the prevention and treatment options of bronchiectasis, including the possible benefit of IGRT. Asymptomatic patients with bronchiectasis and fibrosis-like changes detected on imaging should be followed-up to investigate the progression of lung changes. Lung MRI should be repeated to evaluate the applicability of this imaging modality in the follow-up of pulmonary status.

Future studies should investigate the functional consequences of impaired telomere maintenance in CHH and its potential clinical implications. Assessment of telomere length in children with CHH combined with long-term follow-up would evaluate the applicability of telomere length as a prognostic factor in CHH.

Further research is required in the selection of patients for HSCT, including the applicability of risk factors detected in our studies. In addition, prospective studies are necessary to evaluate the benefit of early HSCT in mildly or moderately symptomatic CHH patients with positive newborn screening for SCID.

Before applying the risk factors for adverse outcomes identified in our studies to the general CHH population, the validation of these risk factors is required in Finnish and non-Finnish patients with CHH. Evidence-based guidelines on the management of patients with CHH are urgently needed to optimize treatment decisions and improve survival. Follow-up on the mortality rates after the implementation of the new management recommendations would demonstrate their impact.

9. CONCLUSIONS

Our studies provided valuable insights into clinical and immunological phenotypes of CHH, guiding the follow-up and management of patients with CHH. We presented previously unavailable data on clinical and immunologic features of older adults with CHH. We described novel findings of high prevalence of specific antibody deficiency and various abnormalities in B and T cell subpopulations. We could not identify any significant correlations of clinical features with laboratory parameters, and our data suggest that in some cases management decisions should be based on clinical manifestations.

We demonstrated for the first time the consequences of *RMRP* mutations on telomere functioning by detecting shorter telomeres in patients with CHH, especially children. These findings can explain the pathogenesis of various clinical features in CHH and have important clinical implications.

We reported a high prevalence of bronchiectasis in the general CHH population and showed that pulmonary evaluation was indicated in all, even asymptomatic, patients with CHH. We compared the performance of lung MRI with HRCT and confirmed that MRI can be implicated in the assessment of pulmonary changes in CHH.

We demonstrated that patients with CHH had high mortality not only due to infections and malignancies, but also from respiratory diseases. We provided long-term follow-up data and described several patterns of disease course, including adult-onset immunodeficiency and the development of malignancies in previously asymptomatic patients. These data call for regular follow-up and screening for cancer in all individuals with CHH, irrespective of the degree of immunodeficiency.

Our data on the risk factors for adverse outcomes in CHH provide clinicians with valuable tool for evaluation of prognosis and we suggest implication of our results for the management of Finnish patients with CHH.

10. ACKNOWLEDGMENTS

This study was conducted in 2014-2018 at the Children's Hospital, University of Helsinki and Helsinki University Hospital, and the Folkhälsan Institute of Genetics, Biomedicum, Helsinki, Finland. I am grateful to Jari Petäjä, Director of Children's Hospital, and Professor Anna-Elina Lehesjoki, the research director of the Folkhälsan Institute of Genetics for providing excellent clinical and laboratory research facilities.

Part of this research was undertaken in Malmi District Hospital in Pietarsaari. I am grateful to the Chief of the Hospital Pia-Maria Sjöström for collaboration, to my good colleagues Markus Granholm, Matias Björn and Ann-Charlotte Hanner, as well as to the excellent nurses, for all their support.

During my doctoral studies I have been working as a Fellow in Pediatric Infectious Diseases at the Children's Hospital. I sincerely thank Anne Wikström, Head of Pediatrics and Professor Harri Saxén of the Children's Hospital for providing me with this opportunity.

This doctoral work was supported by the Doctoral School in Health Science at the Faculty of Medicine, University of Helsinki and Helsinki University Hospital. Additional grants were received from The Sigrid Juselius Foundation and The Finnish Medical Society Duodecim.

My one-of-a-kind supervisor Outi Mäkitie, I remember listening to your lecture on CHH and your mentioning of an open doctoral student position. Fortunately, I was lucky not to miss this opportunity. It has been a wonderful and productive time and I feel privileged to learn from you. I am forever thankful for all your time, support, guidance and for being an inspiring role model for me.

I am grateful to my other supervisor Paula Klemetti for all the support. Your kind advice greatly assisted in my career decisions and achievements. You taught me the importance of thorough and deep analysis of the research results, as well as the subtleties of interpersonal and bureaucratic communication.

My thesis committee members, Matti Korppi and Krista Ress, you have shared with me your experience and advice, and I thank you for your time and assistance.

I am thankful to my external reviewers, Leena Kainulainen and Johanna Lempainen from the Turku University Hospital for their valuable comments and suggestions.

My co-authors, Mervi Taskinen, Helena Valta, Mikko Seppänen, Timi Martelius, Sanna Toiviainen-Salo, Merja Kajosaari, Anna Föhr, Minna Pekkinen, Sofie Degerman, Mattias Landfors, Göran Roos, Eero Pukkala and Luigi Notarangelo, your valuable input dramatically improved the content and enhanced the quality of our published studies. I learned so much from you and I am happy to have collaborated with you.

It’s been a pleasure to be a part of our research group and get to know all its wonderful members: Saila Laakso, Petra Loid, Riikka Mäkitie, Pauliina Utriainen and Ilkka Vuorimies, thank you for your support.

I own a special appreciation to our research nurse Eira Leinonen, who took kind care of me in the beginning of my doctoral studies. You patiently guided me and shared priceless tips, facilitating the onset of my research. Our research nurses Nea Boman and Päivi Turunen, you are the best research nurses one could ask for. Your talent of communication with the study participants and your organizational skills hugely assisted this research work.

Our laboratory manager Minna Pekkinen, you have introduced me to the laboratory work and have guided and supported me throughout these years. I admire your calm, respectful and thoughtful attitude. I am thankful to Hanna Hellgren, who patiently taught me all the necessary laboratory techniques, as well as the significance of accuracy and punctuality. I appreciate the help of our laboratory assistants Mira Aronen and Laura Koljonen, as well as Maura Kere and Natalia Kakko.

I am grateful to all the employees of the Folkhälsan Research Center at Biomedicum Helsinki. You created a friendly working environment and readily offered help for a clinician struggling with learning the laboratory rules, equipment and etiquette.

During my studies, I admired the willingness of the patients, their relatives and healthy volunteers to participate in this research and donate samples. I sincerely thank you for your time, patience and valuable input, that all made this doctoral work possible.

I own an endless gratitude to my mother Natalja, my sister Olga and her son Erik. Your love, care and support accompany me in all my journeys.

My dearest husband Mikko – you have inspired, supported and encouraged me in every possible way. Instead of a thousand words – you are the best of the best.

My precious Seela, you were born in the middle of my doctoral studies and changed my world forever. You are my everything and I dedicate this work to you.

Helsinki

April 2019

Svetlana (Kostjukovits) Vakkilainen

11. REFRENCES

Aalto, S. M., K. Linnavuori, H. Peltola, E. Vuori, B. Weissbrich, J. Schubert, L. Hedman and K. Hedman, "Immunoreactivation of Epstein-Barr virus due to cytomegalovirus primary infection," *J Med Virol,* 56, 186-91 (1998).

Accardi, R. and T. Gheit, "Cutaneous HPV and skin cancer," *Presse Med,* 43, e435-43 (2014).

Alder, J. K., V. S. Hanumanthu, M. A. Strong, A. E. DeZern, S. E. Stanley, C. M. Takemoto, L. Danilova, C. D. Applegate, S. G. Bolton, D. W. Mohr, R. A. Brodsky, J. F. Casella, C. W. Greider, J. B. Jackson and M. Armanios, "Diagnostic utility of telomere length testing in a hospital-based setting," *Proc Natl Acad Sci U S A,* 115, E2358-e2365 (2018).

Alter, B. P., N. Giri, S. A. Savage and P. S. Rosenberg, "Cancer in dyskeratosis congenita," *Blood,* 113, 6549-57 (2009).

Ammann, R. A., A. Duppenthaler, J. Bux and C. Aebi, "Granulocyte colony-stimulating factor-responsive chronic neutropenia in cartilage-hair hypoplasia," *J Pediatr Hematol Oncol,* 26, 379-81 (2004).

Anic, G. M., V. K. Sondak, J. L. Messina, N. A. Fenske, J. S. Zager, B. S. Cherpelis, J. H. Lee, W. J. Fulp, P. K. Epling-Burnette, J. Y. Park and D. E. Rollison, "Telomere length and risk of melanoma, squamous cell carcinoma, and basal cell carcinoma," *Cancer Epidemiol,* 37, 434-9 (2013).

Armanios, M. Y., J. J. Chen, J. D. Cogan, J. K. Alder, R. G. Ingersoll, C. Markin, W. E. Lawson, M. Xie, I. Vulto, J. A. Phillips, 3rd, P. M. Lansdorp, C. W. Greider and J. E. Loyd, "Telomerase mutations in families with idiopathic pulmonary fibrosis," *N Engl J Med,* 356, 1317-26 (2007).

Attarbaschi, A., E. Carraro, O. Abla, S. Barzilai-Birenboim, S. Bomken, L. Brugieres, E. Bubanska, B. Burkhardt, A. K. Chiang, M. Csoka, A. Fedorova, J. Jazbec, E. Kabickova, Z. Krenova, J. Lazic, J. Loeffen, G. Mann, F. Niggli, N. Miakova, T. Osumi, L. Ronceray, A. Uyttebroeck, D. Williams, W. Woessmann, G. Wrobel and M. Pillon, "Non-Hodgkin lymphoma and pre-existing conditions: spectrum, clinical characteristics and outcome in 213 children and adolescents," *Haematologica,* 101, 1581-1591 (2016).

Aubert, G., K. A. Strauss, P. M. Lansdorp and N. L. Rider, "Defects in lymphocyte telomere homeostasis contribute to cellular immune phenotype in patients with cartilage-hair hypoplasia," *J Allergy Clin Immunol,* 140, 1120-1129.e1 (2017).

Bacchetta, J., B. Ranchin, A. S. Brunet, R. Bouvier, A. Duquesne, P. Edery, N. Fabien and N. Peretti, "Autoimmune hypoparathyroidism in a 12-year-old girl with McKusick cartilage hair hypoplasia," *Pediatr Nephrol,* 24, 2449-53 (2009).

Bailly-Botuha, C., F. Jaubert, R. A. Taam, L. Galmiche, C. Picard, G. Bellon and J. de Blic, "Diffuse lymphoplasmacytic bronchiolitis in cartilage-hair hypoplasia," *J Pediatr,* 152, 429-33 (2008).

Baradaran-Heravi, A., C. Thiel, A. Rauch, M. Zenker, C. F. Boerkoel and I. Kaitila, "Clinical and genetic distinction of Schimke immuno-osseous dysplasia and cartilage-hair hypoplasia," *Am J Med Genet A,* 146a, 2013-7 (2008).

Baris, S., H. Ercan, H. H. Cagan, A. Ozen, E. Karakoc-Aydiner, C. Ozdemir and N. N. Bahceciler, "Efficacy of intravenous immunoglobulin treatment in children with common variable immunodeficiency," *J Investig Allergol Clin Immunol,* 21, 514-21 (2011).

Barraza-Garcia, J., C. I. Rivera-Pedroza, A. Hisado-Oliva, A. Belinchon-Martinez, L. Sentchordi-Montane, E. L. Duncan, G. R. Clark, A. Del Pozo, K. Ibanez-Garikano, A. Offiah, P. Prieto-Matos, V. Cormier-Daire and K. E. Heath, "Broadening the phenotypic spectrum of POP1-skeletal dysplasias: identification of POP1 mutations in a mild and severe skeletal dysplasia," *Clin Genet,* 92, 91-98 (2017).

Baumann, U., J. M. Routes, P. Soler-Palacin and S. Jolles, "The Lung in Primary Immunodeficiencies: New Concepts in Infection and Inflammation," *Front Immunol,* 9, 1837 (2018).

Beaussant Cohen, S., O. Fenneteau, E. Plouvier, P. S. Rohrlich, G. Daltroff, I. Plantier, A. Dupuy, D. Kerob, B. Beaupain, P. Bordigoni, F. Fouyssac, A. L. Delezoide, G. Devouassoux, J. F. Nicolas, P. Bensaid, Y. Bertrand, K. Balabanian, C. B. Chantelot, F. Bachelerie and J. Donadieu, "Description and outcome of a cohort of 8 patients with WHIM syndrome from the French Severe Chronic Neutropenia Registry," *Orphanet J Rare Dis,* 7, 71 (2012).

Bertuch, A. A., "The molecular genetics of the telomere biology disorders," *RNA Biol,* 13, 696-706 (2016).

Biederer, J., S. Mirsadraee, M. Beer, F. Molinari, C. Hintze, G. Bauman, M. Both, E. J. Van Beek, J. Wild and M. Puderbach, "MRI of the lung (3/3)-current applications and future perspectives," *Insights Imaging,* 3, 373-86 (2012).

Biggs, C. M., S. Kostjukovits, K. Dobbs, S. Laakso, P. Klemetti, H. Valta, M. Taskinen, O. Makitie and L. D. Notarangelo, "Diverse Autoantibody Reactivity in Cartilage-Hair Hypoplasia," *J Clin Immunol,* 37, 508-510 (2017).

Blanco, E., M. Perez-Andres, S. Arriba-Mendez, T. Contreras-Sanfeliciano, I. Criado, O. Pelak, A. Serra-Caetano, A. Romero, N. Puig, A. Remesal, J. Torres Canizales, E. Lopez-Granados, T. Kalina, A. E. Sousa, M. van Zelm, M. van der Burg, J. J. M. van Dongen and A. Orfao, "Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood," *J Allergy Clin Immunol,* 141, 2208-2219.e16 (2018).

Boldt, A., S. Borte, S. Fricke, K. Kentouche, F. Emmrich, M. Borte, F. Kahlenberg and U. Sack, "Eight-color immunophenotyping of T-, B-, and NK-cell subpopulations for characterization of chronic immunodeficiencies," *Cytometry B Clin Cytom,* 86, 191-206 (2014).

Bonafe, L., E. T. Dermitzakis, S. Unger, C. R. Greenberg, B. A. Campos-Xavier, A. Zankl, C. Ucla, S. E. Antonarakis, A. Superti-Furga and A. Reymond, "Evolutionary comparison provides evidence for pathogenicity of RMRP mutations," *PLoS Genet,* 1, e47 (2005).

Bonafe, L., K. Schmitt, G. Eich, A. Giedion and A. Superti-Furga, "RMRP gene sequence analysis confirms a cartilage-hair hypoplasia variant with only skeletal manifestations and reveals a high density of single-nucleotide polymorphisms," *Clin Genet,* 61, 146-51 (2002).

Bordon, V., A. R. Gennery, M. A. Slatter, E. Vandecruys, G. Laureys, P. Veys, W. Qasim, W. Friedrich, N. M. Wulfraat, F. Scherer, A. J. Cant, A. Fischer, M. Cavazzana-Calvo, R. G. Bredius, L. D. Notarangelo, E. Mazzolari, B. Neven and T. Gungor, "Clinical and immunologic outcome of patients with cartilage hair hypoplasia after hematopoietic stem cell transplantation," *Blood,* 116, 27-35 (2010).

Bousfiha, A., L. Jeddane, C. Picard, F. Ailal, H. Bobby Gaspar, W. Al-Herz, T. Chatila, Y. J. Crow, C. Cunningham-Rundles, A. Etzioni, J. L. Franco, S. M. Holland, C. Klein, T. Morio, H. D. Ochs, E. Oksenhendler, J. Puck, M. L. K. Tang, S. G. Tangye, T. R.

Torgerson, J. L. Casanova and K. E. Sullivan, "The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies," *J Clin Immunol,* 38, 129-143 (2018).
Briggs, T. A., G. I. Rice, N. Adib, L. Ades, S. Barete, K. Baskar, V. Baudouin, A. N. Cebeci, P. Clapuyt, D. Coman, L. De Somer, Y. Finezilber, M. Frydman, A. Guven, S. Heritier, D. Karall, M. L. Kulkarni, P. Lebon, D. Levitt, M. Le Merrer, A. Linglart, J. H. Livingston, V. Navarro, E. Okenfuss, A. Puel, N. Revencu, S. Scholl-Burgi, M. Vivarelli, C. Wouters, B. Bader-Meunier and Y. J. Crow, "Spondyloenchondrodysplasia Due to Mutations in ACP5: A Comprehensive Survey," *J Clin Immunol,* 36, 220-34 (2016).
Calado, R. T. and D. V. Cle, "Treatment of inherited bone marrow failure syndromes beyond transplantation," *Hematology Am Soc Hematol Educ Program,* 2017, 96-101 (2017).
Cawthon, R. M., "Telomere measurement by quantitative PCR," *Nucleic Acids Res,* 30, e47 (2002).
Chandesris, M. O., I. Melki, A. Natividad, A. Puel, C. Fieschi, L. Yun, C. Thumerelle, E. Oksenhendler, D. Boutboul, C. Thomas, C. Hoarau, Y. Lebranchu, J. L. Stephan, C. Cazorla, N. Aladjidi, M. Micheau, F. Tron, A. Baruchel, V. Barlogis, G. Palenzuela, C. Mathey, S. Dominique, G. Body, M. Munzer, F. Fouyssac, R. Jaussaud, B. Bader-Meunier, N. Mahlaoui, S. Blanche, M. Debre, M. Le Bourgeois, V. Gandemer, N. Lambert, V. Grandin, S. Ndaga, C. Jacques, C. Harre, M. Forveille, M. A. Alyanakian, A. Durandy, C. Bodemer, F. Suarez, O. Hermine, O. Lortholary, J. L. Casanova, A. Fischer and C. Picard, "Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey," *Medicine (Baltimore),* 91, e1-19 (2012).
Chang, A. B., A. Bush and K. Grimwood, "Bronchiectasis in children: diagnosis and treatment," *Lancet,* 392, 866-879 (2018).
Daheshia, M., J. D. Prahl, J. J. Carmichael, J. S. Parrish and G. Seda, "The immune response and its therapeutic modulation in bronchiectasis," *Pulm Med,* 2012, 280528 (2012).
de la Fuente, M. A., M. Recher, N. L. Rider, K. A. Strauss, D. H. Morton, M. Adair, F. A. Bonilla, H. D. Ochs, E. W. Gelfand, I. M. Pessach, J. E. Walter, A. King, S. Giliani, S. Y. Pai and L. D. Notarangelo, "Reduced thymic output, cell cycle abnormalities, and increased apoptosis of T lymphocytes in patients with cartilage-hair hypoplasia," *J Allergy Clin Immunol,* 128, 139-146 (2011).
Degerman, S., M. Domellof, M. Landfors, J. Linder, M. Lundin, S. Haraldsson, E. Elgh, G. Roos and L. Forsgren, "Long leukocyte telomere length at diagnosis is a risk factor for dementia progression in idiopathic parkinsonism," *PLoS One,* 9, e113387 (2014).
Dinur Schejter, Y., A. Ovadia, R. Alexandrova, B. Thiruvahindrapuram, S. L. Pereira, D. E. Manson, A. Vincent, D. Merico and C. M. Roifman, "A homozygous mutation in the stem II domain of RNU4ATAC causes typical Roifman syndrome," *NPJ Genom Med,* 2, 23 (2017).
Driessen, G. J., M. C. van Zelm, P. M. van Hagen, N. G. Hartwig, M. Trip, A. Warris, E. de Vries, B. H. Barendregt, I. Pico, W. Hop, J. J. van Dongen and M. van der Burg, "B-cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency," *Blood,* 118, 6814-23 (2011).
Eisner, J. M. and M. Russell, "Cartilage hair hypoplasia and multiple basal cell carcinomas," *J Am Acad Dermatol,* 54, S8-10 (2006).
Elalaoui, S. C., F. Z. Laarabi, M. Mansouri, N. A. Mrani, G. Nishimura and A. Sefiani, "Further evidence of POP1 mutations as the cause of anauxetic dysplasia," *Am J Med Genet A,* 170, 2462-5 (2016).

Esakova, O. and A. S. Krasilnikov, "Of proteins and RNA: the RNase P/MRP family," *Rna,* 16, 1725-47 (2010).
Farach, L. S., M. E. Little, A. L. Duker, C. V. Logan, A. Jackson, J. T. Hecht and M. Bober, "The expanding phenotype of RNU4ATAC pathogenic variants to Lowry Wood syndrome," *Am J Med Genet A,* 176, 465-469 (2018).
Flume, P. A., J. D. Chalmers and K. N. Olivier, "Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity," *Lancet,* 392, 880-890 (2018).
Fox, T. A., R. Chakraverty, S. Burns, B. Carpenter, K. Thomson, D. Lowe, A. Fielding, K. Peggs, P. Kottaridis, B. Uttenthal, V. Bigley, M. Buckland, V. Grandage, S. Denovan, S. Grace, J. Dahlstrom, S. Workman, A. Symes, S. Mackinnon, R. Hough and E. Morris, "Successful outcome following allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency," *Blood,* 131, 917-931 (2018).
Glazov, E. A., A. Zankl, M. Donskoi, T. J. Kenna, G. P. Thomas, G. R. Clark, E. L. Duncan and M. A. Brown, "Whole-exome re-sequencing in a family quartet identifies POP1 mutations as the cause of a novel skeletal dysplasia," *PLoS Genet,* 7, e1002027 (2011).
Gosain, A. and A. S. Brinkman, "Hirschsprung's associated enterocolitis," *Curr Opin Pediatr,* 27, 364-9 (2015).
Gregersen, S., T. M. Aalokken, G. Mynarek, B. Fevang, A. M. Holm, T. Ueland, P. Aukrust, J. Kongerud, B. Johansen and S. S. Froland, "Development of pulmonary abnormalities in patients with common variable immunodeficiency: associations with clinical and immunologic factors," *Ann Allergy Asthma Immunol,* 104, 503-10 (2010).
Guggenheim, R., R. Somech, E. Grunebaum, A. Atkinson and C. M. Roifman, "Bone marrow transplantation for cartilage-hair-hypoplasia," *Bone Marrow Transplant,* 38, 751-6 (2006).
Guilemany, J. M., J. Angrill, I. Alobid, S. Centellas, E. Prades, J. Roca, L. Pujols, M. Bernal-Sprekelsen, C. Picado and J. Mullol, "United airways: the impact of chronic rhinosinusitis and nasal polyps in bronchiectasic patient's quality of life," *Allergy,* 64, 1524-9 (2009).
Guo, L., N. H. Elcioglu, S. Mizumoto, Z. Wang, B. Noyan, H. M. Albayrak, S. Yamada, N. Matsumoto, N. Miyake, G. Nishimura and S. Ikegawa, "Identification of biallelic EXTL3 mutations in a novel type of spondylo-epi-metaphyseal dysplasia," *J Hum Genet,* 62, 797-801 (2017).
Harley, C. B., A. B. Futcher and C. W. Greider, "Telomeres shorten during ageing of human fibroblasts," *Nature,* 345, 458-60 (1990).
Hauck, F., R. Voss, C. Urban and M. G. Seidel, "Intrinsic and extrinsic causes of malignancies in patients with primary immunodeficiency disorders," *J Allergy Clin Immunol,* 141, 59-68.e4 (2018).
Heremans, J., J. E. Garcia-Perez, E. Turro, S. M. Schlenner, I. Casteels, R. Collin, F. de Zegher, D. Greene, S. Humblet-Baron, S. Lesage, P. Matthys, C. J. Penkett, K. Put, K. Stirrups, C. Thys, C. Van Geet, E. Van Nieuwenhove, C. Wouters, I. Meyts, K. Freson and A. Liston, "Abnormal differentiation of B cells and megakaryocytes in patients with Roifman syndrome," *J Allergy Clin Immunol,* 142, 630-646 (2018).
Hermanns, P., A. Tran, E. Munivez, S. Carter, B. Zabel, B. Lee and J. G. Leroy, "RMRP mutations in cartilage-hair hypoplasia," *Am J Med Genet A,* 140, 2121-30 (2006).
Horn, D., E. Rupprecht, J. Kunze and J. Spranger, "Anauxetic dysplasia, a spondylometaepiphyseal dysplasia with extreme dwarfism," *J Med Genet,* 38, 262-5 (2001).

Horn, J., M. Schlesier, K. Warnatz, A. Prasse, A. Superti-Furga, H. H. Peter and U. Salzer, "Fatal adult-onset antibody deficiency syndrome in a patient with cartilage hair hypoplasia," *Hum Immunol,* 71, 916-9 (2010).
Ip, W., H. B. Gaspar, R. Kleta, E. Chanudet, C. Bacchelli, A. Pitts, Z. Nademi, E. G. Davies, M. A. Slatter, P. Amrolia, K. Rao, P. Veys, A. R. Gennery and W. Qasim, "Variable phenotype of severe immunodeficiencies associated with RMRP gene mutations," *J Clin Immunol,* 35, 147-57 (2015).
Jaaskelainen, A. J., S. M. Viitala, S. Kurkela, S. Hepojoki, H. Sillanpaa, H. Kallio-Kokko, T. Bergstrom, J. Suni, A. Narvanen, O. Vapalahti and A. Vaheri, "Performance of a multiplexed serological microarray for the detection of antibodies against central nervous system pathogens," *J Microbiol Methods,* 100, 27-31 (2014).
Juvonen, E., O. Makitie, A. Makipernaa, T. Ruutu, I. Kaitila and J. Rajantie, "Defective in-vitro colony formation of haematopoietic progenitors in patients with cartilage-hair hypoplasia and history of anaemia," *Eur J Pediatr,* 154, 30-4 (1995).
Kainulainen, L., O. Lassila and O. Ruuskanen, "Cartilage-hair hypoplasia: follow-up of immunodeficiency in two patients," *J Clin Immunol,* 34, 256-9 (2014).
Kamani, N. R., S. Kumar, A. Hassebroek, M. Eapen, J. LeRademacher, J. Casper, M. Cowan, J. Sanchez de Toledo, A. Ferster, P. Szabolcs, J. R. Wingard, E. Horwitz and A. H. Filipovich, "Malignancies after hematopoietic cell transplantation for primary immune deficiencies: a report from the Center for International Blood and Marrow Transplant Research," *Biol Blood Marrow Transplant,* 17, 1783-9 (2011).
Kavadas, F. D., S. Giliani, Y. Gu, E. Mazzolari, A. Bates, E. Pegoiani, C. M. Roifman and L. D. Notarangelo, "Variability of clinical and laboratory features among patients with ribonuclease mitochondrial RNA processing endoribonuclease gene mutations," *J Allergy Clin Immunol,* 122, 1178-84 (2008).
Kersseboom, R., A. Brooks and C. Weemaes, "Educational paper: syndromic forms of primary immunodeficiency," *Eur J Pediatr,* 170, 295-308 (2011).
Klemetti, P., H. Valta, S. Kostjukovits, M. Taskinen, S. Toiviainen-Salo and O. Makitie, "Cartilage-hair hypoplasia with normal height in childhood-4 patients with a unique genotype," *Clin Genet,* 92, 204-207 (2017).
Kotaniemi, J. T., P. Pallasaho, A. R. Sovijarvi, L. A. Laitinen and B. Lundback, "Respiratory symptoms and asthma in relation to cold climate, inhaled allergens, and irritants: a comparison between northern and southern Finland," *J Asthma,* 39, 649-58 (2002).
Kristiansen, M., H. Aggerbeck and I. Heron, "Improved ELISA for determination of anti-diphtheria and/or anti-tetanus antitoxin antibodies in sera," *Apmis,* 105, 843-53 (1997).
Kroigard, A. B., M. Frost, M. J. Larsen, L. B. Ousager and A. L. Frederiksen, "Bone structure in two adult subjects with impaired minor spliceosome function resulting from RNU4ATAC mutations causing microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1)," *Bone,* 92, 145-149 (2016).
Kwak, H. J., J. Y. Moon, Y. W. Choi, T. H. Kim, J. W. Sohn, H. J. Yoon, D. H. Shin, S. S. Park and S. H. Kim, "High prevalence of bronchiectasis in adults: analysis of CT findings in a health screening program," *Tohoku J Exp Med,* 222, 237-42 (2010).
Leiding, J. W. and L. R. Forbes, "Mechanism-Based Precision Therapy for the Treatment of Primary Immunodeficiency and Primary Immunodysregulatory Diseases," *J Allergy Clin Immunol Pract,* 7, 761-773 (2019).

Leiding, J. W. and S. M. Holland, "Warts and all: human papillomavirus in primary immunodeficiencies," *J Allergy Clin Immunol,* 130, 1030-48 (2012).
Lipska-Zietkiewicz, B. S., J. Gellermann, O. Boyer, O. Gribouval, S. Zietkiewicz, J. A. Kari, M. A. Shalaby, F. Ozaltin, J. Dusek, A. Melk, A. K. Bayazit, L. Massella, L. Hyla-Klekot, S. Habbig, A. Godron, M. Szczepanska, B. Bienias, D. Drozdz, R. Odeh, W. Jarmuzek, K. Zachwieja, A. Trautmann, C. Antignac and F. Schaefer, "Low renal but high extrarenal phenotype variability in Schimke immuno-osseous dysplasia," *PLoS One,* 12, e0180926 (2017).
Lux, S. E., R. B. Johnston, Jr., C. S. August, B. Say, V. B. Penchaszadeh, F. S. Rosen and V. A. McKusick, "Chronic neutropenia and abnormal cellular immunity in cartilage-hair hypoplasia," *N Engl J Med,* 282, 231-6 (1970).
Maffucci, P., J. Chavez, T. J. Jurkiw, P. J. O'Brien, J. K. Abbott, P. R. Reynolds, A. Worth, L. D. Notarangelo, K. Felgentreff, P. Cortes, B. Boisson, L. Radigan, A. Cobat, C. Dinakar, M. Ehlayel, T. Ben-Omran, E. W. Gelfand, J. L. Casanova and C. Cunningham-Rundles, "Biallelic mutations in DNA ligase 1 underlie a spectrum of immune deficiencies," *J Clin Invest,* 128, 5489-5504 (2018).
Maida, Y., M. Yasukawa, M. Furuuchi, T. Lassmann, R. Possemato, N. Okamoto, V. Kasim, Y. Hayashizaki, W. C. Hahn and K. Masutomi, "An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA," *Nature,* 461, 230-5 (2009).
Makitie, O., "Cartilage-hair hypoplasia in Finland: epidemiological and genetic aspects of 107 patients," *J Med Genet,* 29, 652-5 (1992).
Makitie, O., M. Heikkinen, I. Kaitila and R. Rintala, "Hirschsprung's disease in cartilage-hair hypoplasia has poor prognosis," *J Pediatr Surg,* 37, 1585-8 (2002).
Makitie, O. and I. Kaitila, "Cartilage-hair hypoplasia--clinical manifestations in 108 Finnish patients," *Eur J Pediatr,* 152, 211-7 (1993).
Makitie, O., I. Kaitila and R. Rintala, "Hirschsprung disease associated with severe cartilage-hair hypoplasia," *J Pediatr,* 138, 929-31 (2001).
Makitie, O., I. Kaitila and E. Savilahti, "Susceptibility to infections and in vitro immune functions in cartilage-hair hypoplasia," *Eur J Pediatr,* 157, 816-20 (1998).
Makitie, O., I. Kaitila and E. Savilahti, "Deficiency of humoral immunity in cartilage-hair hypoplasia," *J Pediatr,* 137, 487-92 (2000).
Makitie, O., J. Perheentupa and I. Kaitila, "Growth in cartilage-hair hypoplasia," *Pediatr Res,* 31, 176-80 (1992).
Makitie, O., E. Pukkala and I. Kaitila, "Increased mortality in cartilage-hair hypoplasia," *Arch Dis Child,* 84, 65-67 (2001).
Makitie, O., E. Pukkala, L. Teppo and I. Kaitila, "Increased incidence of cancer in patients with cartilage-hair hypoplasia," *J Pediatr,* 134, 315-8 (1999).
Makitie, O., J. Rajantie and I. Kaitila, "Anaemia and macrocytosis--unrecognized features in cartilage-hair hypoplasia," *Acta Paediatr,* 81, 1026-9 (1992).
Maroteaux, S. P., P. Savart, J. Lefebvre and P. Royer, "Les formes partielles de la dysostose metaphysaire," *Presse Med*, 1523-6 (1963).
Martin, A. N. and Y. Li, "RNase MRP RNA and human genetic diseases," *Cell Res,* 17, 219-26 (2007).
Mattijssen, S., T. J. Welting and G. J. Pruijn, "RNase MRP and disease," *Wiley Interdiscip Rev RNA,* 1, 102-16 (2010).

Mayor, P. C., K. H. Eng, K. L. Singel, S. I. Abrams, K. Odunsi, K. B. Moysich, R. Fuleihan, E. Garabedian, P. Lugar, H. D. Ochs, F. A. Bonilla, R. H. Buckley, K. E. Sullivan, Z. K. Ballas, C. Cunningham-Rundles and B. H. Segal, "Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry," *J Allergy Clin Immunol,* 141, 1028-1035 (2018).

McCann, L. J., J. McPartland, D. Barge, L. Strain, D. Bourn, E. Calonje, J. Verbov, A. Riordan, G. Kokai, C. M. Bacon, M. Wright and M. Abinun, "Phenotypic variations of cartilage hair hypoplasia: granulomatous skin inflammation and severe T cell immunodeficiency as initial clinical presentation in otherwise well child with short stature," *J Clin Immunol,* 34, 42-8 (2014).

McCusker, C., J. Upton and R. Warrington, "Primary immunodeficiency," *Allergy Asthma Clin Immunol,* 14, 61 (2018).

McKusick, V. A., R. Eldridge, J. A. Hostetler, U. Ruangwit and J. A. Egeland, "DWARFISM IN THE AMISH. II. CARTILAGE-HAIR HYPOPLASIA," *Bull Johns Hopkins Hosp,* 116, 285-326 (1965).

Menger, H., S. Mundlos, K. Becker, J. Spranger and B. Zabel, "An unknown spondylo-meta-epiphyseal dysplasia in sibs with extreme short stature," *Am J Med Genet,* 63, 80-3 (1996).

Merico, D., M. Roifman, U. Braunschweig, R. K. Yuen, R. Alexandrova, A. Bates, B. Reid, T. Nalpathamkalam, Z. Wang, B. Thiruvahindrapuram, P. Gray, A. Kakakios, J. Peake, S. Hogarth, D. Manson, R. Buncic, S. L. Pereira, J. A. Herbrick, B. J. Blencowe, C. M. Roifman and S. W. Scherer, "Compound heterozygous mutations in the noncoding RNU4ATAC cause Roifman Syndrome by disrupting minor intron splicing," *Nat Commun,* 6, 8718 (2015).

Milanowska, K., K. Mikolajczak, A. Lukasik, M. Skorupski, Z. Balcer, M. A. Machnicka, M. Nowacka, K. M. Rother and J. M. Bujnicki, "RNApathwaysDB--a database of RNA maturation and decay pathways," *Nucleic Acids Res,* 41, D268-72 (2013).

Milito, C., F. Pulvirenti, G. Serra, M. Valente, A. M. Pesce, G. Granata, C. Catalano, F. Fraioli and I. Quinti, "Lung magnetic resonance imaging with diffusion weighted imaging provides regional structural as well as functional information without radiation exposure in primary antibody deficiencies," *J Clin Immunol,* 35, 491-500 (2015).

Montella, S., M. Maglione, D. Bruzzese, C. Mollica, C. Pignata, G. Aloj, A. Manna, A. Esposito, V. Mirra and F. Santamaria, "Magnetic resonance imaging is an accurate and reliable method to evaluate non-cystic fibrosis paediatric lung disease," *Respirology,* 17, 87-91 (2012).

Morin, G. B., "The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats," *Cell,* 59, 521-9 (1989).

Mortaz, E., P. Tabarsi, D. Mansouri, A. Khosravi, J. Garssen, A. Velayati and I. M. Adcock, "Cancers Related to Immunodeficiencies: Update and Perspectives," *Front Immunol,* 7, 365 (2016).

Moshous, D., I. Meyts, S. Fraitag, C. E. Janssen, M. Debre, F. Suarez, J. Toelen, K. De Boeck, T. Roskams, A. Deschildre, C. Picard, C. Bodemer, C. Wouters and A. Fischer, "Granulomatous inflammation in cartilage-hair hypoplasia: risks and benefits of anti-TNF-alpha mAbs," *J Allergy Clin Immunol,* 128, 847-53 (2011).

Nelson, N. D. and A. A. Bertuch, "Dyskeratosis congenita as a disorder of telomere maintenance," *Mutat Res,* 730, 43-51 (2012).

Nguyen, A., N. Martin Silva, H. de Boysson, G. Damaj and A. Aouba, "Diffuse large B-cell lymphoma chemotherapy reveals a combined immunodeficiency syndrome in cartilage hair hypoplasia," *Swiss Med Wkly,* 148, w14606 (2018).

Noelle, R. J. and E. C. Snow, "T helper cell-dependent B cell activation," *Faseb j,* 5, 2770-6 (1991).

Notarangelo, L. D., "Expanding the spectrum of skeletal dysplasia with immunodeficiency: a commentary on identification of biallelic EXTL3 mutations in a novel type of spondylo-epi-metaphyseal dysplasia," *J Hum Genet,* 62, 737-738 (2017).

Notarangelo, L. D., C. M. Roifman and S. Giliani, "Cartilage-hair hypoplasia: molecular basis and heterogeneity of the immunological phenotype," *Curr Opin Allergy Clin Immunol,* 8, 534-9 (2008).

Ochs, H. D. and D. Petroni, "From clinical observations and molecular dissection to novel therapeutic strategies for primary immunodeficiency disorders," *Am J Med Genet A,* 176, 784-803 (2018).

Oud, M. M., P. Tuijnenburg, M. Hempel, N. van Vlies, Z. Ren, S. Ferdinandusse, M. H. Jansen, R. Santer, J. Johannsen, C. Bacchelli, M. Alders, R. Li, R. Davies, L. Dupuis, C. M. Cale, R. J. A. Wanders, S. T. Pals, L. Ocaka, C. James, I. Muller, K. Lehmberg, T. Strom, H. Engels, H. J. Williams, P. Beales, R. Roepman, P. Dias, H. G. Brunner, J. M. Cobben, C. Hall, T. Hartley, P. Le Quesne Stabej, R. Mendoza-Londono, E. G. Davies, S. B. de Sousa, D. Lessel, H. H. Arts and T. W. Kuijpers, "Mutations in EXTL3 Cause Neuro-immuno-skeletal Dysplasia Syndrome," *Am J Hum Genet,* 100, 281-296 (2017).

Owayed, A. and W. Al-Herz, "Sinopulmonary Complications in Subjects With Primary Immunodeficiency," *Respir Care,* 61, 1067-72 (2016).

Pacheco-Cuellar, G., J. Gauthier, V. Desilets, C. Lachance, M. Lemire-Girard, F. Rypens, F. Le Deist, H. Decaluwe, M. Duval, D. Bouron-Dal Soglio, V. Kokta, E. Haddad and P. M. Campeau, "A Novel PGM3 Mutation Is Associated With a Severe Phenotype of Bone Marrow Failure, Severe Combined Immunodeficiency, Skeletal Dysplasia, and Congenital Malformations," *J Bone Miner Res,* 32, 1853-1859 (2017).

Perheentupa, J., "[3 hereditary growth disorders]," *Duodecim,* 88, 60-71 (1972).

Picard, C., H. Bobby Gaspar, W. Al-Herz, A. Bousfiha, J. L. Casanova, T. Chatila, Y. J. Crow, C. Cunningham-Rundles, A. Etzioni, J. L. Franco, S. M. Holland, C. Klein, T. Morio, H. D. Ochs, E. Oksenhendler, J. Puck, M. L. K. Tang, S. G. Tangye, T. R. Torgerson and K. E. Sullivan, "International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity," *J Clin Immunol,* 38, 96-128 (2018).

Pierce, G. F., C. Brovall, B. Z. Schacter and S. H. Polmar, "Impaired culture generated cytotoxicity with preservation of spontaneous natural killer-cell activity in cartilage-hair hypoplasia," *J Clin Invest,* 71, 1737-43 (1983).

Pierce, G. F. and S. H. Polmar, "Lymphocyte dysfunction in cartilage-hair hypoplasia: evidence for an intrinsic defect in cellular proliferation," *J Immunol,* 129, 570-5 (1982).

Pihkala, J., T. Hakala, P. Voutilainen and K. Raivio, "[Characteristic of recent fetal growth curves in Finland]," *Duodecim,* 105, 1540-6 (1989).

Puderbach, M., M. Eichinger, J. Haeselbarth, S. Ley, A. Kopp-Schneider, S. Tuengerthal, A. Schmaehl, C. Fink, C. Plathow, M. Wiebel, S. Demirakca, F. M. Muller and H. U. Kauczor, "Assessment of morphological MRI for pulmonary changes in cystic fibrosis (CF) patients: comparison to thin-section CT and chest x-ray," *Invest Radiol,* 42, 715-25 (2007).

Quinti, I., A. Soresina, A. Guerra, R. Rondelli, G. Spadaro, C. Agostini, C. Milito, A. C. Trombetta, M. Visentini, H. Martini, A. Plebani and M. Fiorilli, "Effectiveness of immunoglobulin replacement therapy on clinical outcome in patients with primary antibody deficiencies: results from a multicenter prospective cohort study," *J Clin Immunol,* 31, 315-22 (2011).

Ranki, A., J. Perheentupa, L. C. Andersson and P. Hayry, "In vitro T- and B-cell reactivity in cartilage hair hypoplasia," *Clin Exp Immunol,* 32, 352-60 (1978).

Reisi, M., G. Azizi, F. Kiaee, F. Masiha, R. Shirzadi, T. Momen, H. Rafiemanesh, N. Tavakolinia, M. Modaresi and A. Aghamohammadi, "Evaluation of pulmonary complications in patients with primary immunodeficiency disorders," *Eur Ann Allergy Clin Immunol,* 49, 122-128 (2017).

Remes, S. T., M. Korppi, M. Kajosaari, A. Koivikko, L. Soininen and J. Pekkanen, "Prevalence of allergic rhinitis and atopic dermatitis among children in four regions of Finland," *Allergy,* 53, 682-9 (1998).

Rezaei, N., A. Aghamohammadi, M. Moin, Z. Pourpak, M. Movahedi, M. Gharagozlou, L. Atarod, B. M. Ghazi, A. Isaeian, M. Mahmoudi, K. Abolmaali, D. Mansouri, S. Arshi, N. J. Tarash, R. Sherkat, H. Akbari, R. Amin, A. Alborzi, S. Kashef, R. Farid, I. Mohammadzadeh, M. S. Shabestari, M. Nabavi and A. Farhoudi, "Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry," *J Clin Immunol,* 26, 519-32 (2006).

Ridanpaa, M., P. Jain, V. A. McKusick, C. A. Francomano and I. Kaitila, "The major mutation in the RMRP gene causing CHH among the Amish is the same as that found in most Finnish cases," *Am J Med Genet C Semin Med Genet,* 121c, 81-3 (2003).

Ridanpaa, M., P. Sistonen, S. Rockas, D. L. Rimoin, O. Makitie and I. Kaitila, "Worldwide mutation spectrum in cartilage-hair hypoplasia: ancient founder origin of the major70A-->G mutation of the untranslated RMRP," *Eur J Hum Genet,* 10, 439-47 (2002).

Ridanpaa, M., H. van Eenennaam, K. Pelin, R. Chadwick, C. Johnson, B. Yuan, W. vanVenrooij, G. Pruijn, R. Salmela, S. Rockas, O. Makitie, I. Kaitila and A. de la Chapelle, "Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia," *Cell,* 104, 195-203 (2001).

Rider, N. L., D. H. Morton, E. Puffenberger, C. L. Hendrickson, D. L. Robinson and K. A. Strauss, "Immunologic and clinical features of 25 Amish patients with RMRP 70 A-->G cartilage hair hypoplasia," *Clin Immunol,* 131, 119-28 (2009).

Riley, P., Jr., D. S. Weiner, B. Leighley, D. Jonah, D. H. Morton, K. A. Strauss, M. B. Bober and M. S. Dicintio, "Cartilage hair hypoplasia: characteristics and orthopaedic manifestations," *J Child Orthop,* 9, 145-52 (2015).

Rogler, L. E., B. Kosmyna, D. Moskowitz, R. Bebawee, J. Rahimzadeh, K. Kutchko, A. Laederach, L. D. Notarangelo, S. Giliani, E. Bouhassira, P. Frenette, J. Roy-Chowdhury and C. E. Rogler, "Small RNAs derived from lncRNA RNase MRP have gene-silencing activity relevant to human cartilage-hair hypoplasia," *Hum Mol Genet,* 23, 368-82 (2014).

Roifman, C. M., "Antibody deficiency, growth retardation, spondyloepiphyseal dysplasia and retinal dystrophy: a novel syndrome," *Clin Genet,* 55, 103-9 (1999).

Roifman, C. M., Y. Gu and A. Cohen, "Mutations in the RNA component of RNase mitochondrial RNA processing might cause Omenn syndrome," *J Allergy Clin Immunol,* 117, 897-903 (2006).

Roifman, C. M. and I. Melamed, "A novel syndrome of combined immunodeficiency, autoimmunity and spondylometaphyseal dysplasia," *Clin Genet,* 63, 522-9 (2003).
Sanchez-Ramon, S., A. Bermudez, L. I. Gonzalez-Granado, C. Rodriguez-Gallego, A. Sastre and P. Soler-Palacin, "Primary and Secondary Immunodeficiency Diseases in Oncohaematology: Warning Signs, Diagnosis, and Management," *Front Immunol,* 10, 586 (2019).
Sathishkumar, D., J. E. Gach, M. Ogboli, M. Desai, T. Cole, W. Hogler, J. Motwani, A. Norton, B. Morland and I. Colmenero, "Cartilage hair hypoplasia with cutaneous lymphomatoid granulomatosis," *Clin Exp Dermatol,* 43, 713-717 (2018).
Savage, S. A., "Dyskeratosis Congenita," in *GeneReviews((R))*, M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens and A. Amemiya, Eds., ed., p.^pp., University of Washington, Seattle

University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved., Seattle (WA) (1993).
Schatorje, E. J., E. F. Gemen, G. J. Driessen, J. Leuvenink, R. W. van Hout and E. de Vries, "Paediatric reference values for the peripheral T cell compartment," *Scand J Immunol,* 75, 436-44 (2012).
Schimke, R. N., W. A. Horton, C. R. King and N. L. Martin, "Chondroitin-6-sulfate mucopoly-saccharidosis in conjunction with lymphopenia, defective cellular immunity and the nephrotic syndrome," *Birth Defects Orig Artic Ser,* 10, 258-66 (1974).
Shearer, W. T., H. M. Rosenblatt, R. S. Gelman, R. Oyomopito, S. Plaeger, E. R. Stiehm, D. W. Wara, S. D. Douglas, K. Luzuriaga, E. J. McFarland, R. Yogev, M. H. Rathore, W. Levy, B. L. Graham and S. A. Spector, "Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study," *J Allergy Clin Immunol,* 112, 973-80 (2003).
Shelihan, I., S. Ehresmann, C. Magnani, F. Forzano, C. Baldo, N. Brunetti-Pierri and P. M. Campeau, "Lowry-Wood syndrome: further evidence of association with RNU4ATAC, and correlation between genotype and phenotype," *Hum Genet,* 137, 905-909 (2018).
Sileo, C., H. Corvol, P. Y. Boelle, E. Blondiaux, A. Clement and H. Ducou Le Pointe, "HRCT and MRI of the lung in children with cystic fibrosis: comparison of different scoring systems," *J Cyst Fibros,* 13, 198-204 (2014).
Smevik, B., "Complementary investigations in bronchiectasis in children," *Monaldi Arch Chest Dis,* 55, 420-6 (2000).
Sorensen, R. U. and L. E. Leiva, "Measurement of pneumococcal polysaccharide antibodies," *J Clin Immunol,* 34, 127-8 (2014).
Stray-Pedersen, A., P. H. Backe, H. S. Sorte, L. Morkrid, N. Y. Chokshi, H. C. Erichsen, T. Gambin, K. B. Elgstoen, M. Bjoras, M. W. Wlodarski, M. Kruger, S. N. Jhangiani, D. M. Muzny, A. Patel, K. M. Raymond, G. S. Sasa, R. A. Krance, C. A. Martinez, S. M. Abraham, C. Speckmann, S. Ehl, P. Hall, L. R. Forbes, E. Merckoll, J. Westvik, G. Nishimura, C. F. Rustad, T. G. Abrahamsen, A. Ronnestad, L. T. Osnes, T. Egeland, O. K. Rodningen, C. R. Beck, E. A. Boerwinkle, R. A. Gibbs, J. R. Lupski, J. S. Orange, E. Lausch and I. C. Hanson, "PGM3 mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia," *Am J Hum Genet,* 95, 96-107 (2014).
Sullivan KE, S. E. e., "Hypogammaglobulinemia and Common Variable Immunodeficiency," in *Stiehm's Immune Deficiencies*, 1st edition ed., p.^pp., Academic Press, London (2014).

Taskinen, M., L. Jeskanen, M. L. Karjalainen-Lindsberg, A. Makitie, O. Makitie and A. Ranki, "Combating cancer predisposition in association with idiopathic immune deficiency: a recurrent nodal and cutaneous T-cell lymphoproliferative disease in a patient with cartilage-hair hypoplasia," *Clin Lymphoma Myeloma Leuk,* 13, 73-6 (2013).
Taskinen, M., A. Ranki, E. Pukkala, L. Jeskanen, I. Kaitila and O. Makitie, "Extended follow-up of the Finnish cartilage-hair hypoplasia cohort confirms high incidence of non-Hodgkin lymphoma and basal cell carcinoma," *Am J Med Genet A,* 146a, 2370-5 (2008).
Taskinen, M., S. Toiviainen-Salo, J. Lohi, P. Vuolukka, M. Grasbeck and O. Makitie, "Hypoplastic anemia in cartilage-hair hypoplasia-balancing between iron overload and chelation," *J Pediatr,* 162, 844-9 (2013).
Thickett, K. M., D. S. Kumararatne, A. K. Banerjee, R. Dudley and D. E. Stableforth, "Common variable immune deficiency: respiratory manifestations, pulmonary function and high-resolution CT scan findings," *Qjm,* 95, 655-62 (2002).
Thiel, C. T., D. Horn, B. Zabel, A. B. Ekici, K. Salinas, E. Gebhart, F. Ruschendorf, H. Sticht, J. Spranger, D. Muller, C. Zweier, M. E. Schmitt, A. Reis and A. Rauch, "Severely incapacitating mutations in patients with extreme short stature identify RNA-processing endoribonuclease RMRP as an essential cell growth regulator," *Am J Hum Genet,* 77, 795-806 (2005).
Thiel, C. T., G. Mortier, I. Kaitila, A. Reis and A. Rauch, "Type and level of RMRP functional impairment predicts phenotype in the cartilage hair hypoplasia-anauxetic dysplasia spectrum," *Am J Hum Genet,* 81, 519-29 (2007).
Thiel, C. T. and A. Rauch, "The molecular basis of the cartilage-hair hypoplasia-anauxetic dysplasia spectrum," *Best Pract Res Clin Endocrinol Metab,* 25, 131-42 (2011).
Timby, N., O. Hernell, O. Vaarala, M. Melin, B. Lonnerdal and M. Domellof, "Infections in infants fed formula supplemented with bovine milk fat globule membranes," *J Pediatr Gastroenterol Nutr,* 60, 384-9 (2015).
Todorovic, M., B. Balint, B. Andjelic and B. Mihaljevic, "Multiple malignancies in a female patient with common variable immunodeficiency syndrome," *Singapore Med J,* 55, e162-4 (2014).
Toiviainen-Salo, S., M. Kajosaari, A. Piilonen and O. Makitie, "Patients with cartilage-hair hypoplasia have an increased risk for bronchiectasis," *J Pediatr,* 152, 422-8 (2008).
Wagner, C. L., V. S. Hanumanthu, C. C. Talbot, Jr., R. S. Abraham, D. Hamm, D. L. Gable, C. G. Kanakry, C. D. Applegate, J. Siliciano, J. B. Jackson, S. Desiderio, J. K. Alder, L. Luznik and M. Armanios, "Short telomere syndromes cause a primary T cell immunodeficiency," *J Clin Invest,* (2018).
Vakkilainen, S., R. Makitie, P. Klemetti, H. Valta, M. Taskinen, E. S. Husebye and O. Makitie, "A Wide Spectrum of Autoimmune Manifestations and Other Symptoms Suggesting Immune Dysregulation in Patients With Cartilage-Hair Hypoplasia," *Front Immunol,* 9, 2468 (2018).
van der Burgt, I., A. Haraldsson, J. C. Oosterwijk, A. J. van Essen, C. Weemaes and B. Hamel, "Cartilage hair hypoplasia, metaphyseal chondrodysplasia type McKusick: description of seven patients and review of the literature," *Am J Med Genet,* 41, 371-80 (1991).
Vatanavicharn, N., N. Visitsunthorn, T. Pho-iam, O. Jirapongsananuruk, P. Pacharn, K. Chokephaibulkit, C. Limwongse and P. Wasant, "An infant with cartilage-hair hypoplasia

due to a novel homozygous mutation in the promoter region of the RMRP gene associated with chondrodysplasia and severe immunodeficiency," *J Appl Genet,* 51, 523-8 (2010).
Wehr, C., T. Kivioja, C. Schmitt, B. Ferry, T. Witte, E. Eren, M. Vlkova, M. Hernandez, D. Detkova, P. R. Bos, G. Poerksen, H. von Bernuth, U. Baumann, S. Goldacker, S. Gutenberger, M. Schlesier, F. Bergeron-van der Cruyssen, M. Le Garff, P. Debre, R. Jacobs, J. Jones, E. Bateman, J. Litzman, P. M. van Hagen, A. Plebani, R. E. Schmidt, V. Thon, I. Quinti, T. Espanol, A. D. Webster, H. Chapel, M. Vihinen, E. Oksenhendler, H. H. Peter and K. Warnatz, "The EUROclass trial: defining subgroups in common variable immunodeficiency," *Blood,* 111, 77-85 (2008).
Verhoeven, D., A. J. Stoppelenburg, F. Meyer-Wentrup and M. Boes, "Increased risk of hematologic malignancies in primary immunodeficiency disorders: opportunities for immunotherapy," *Clin Immunol,* 190, 22-31 (2018).
Verloes, A., G. E. Pierard, M. Le Merrer and P. Maroteaux, "Recessive metaphyseal dysplasia without hypotrichosis. A syndrome clinically distinct from McKusick cartilage-hair hypoplasia," *J Med Genet,* 27, 693-6 (1990).
Williams, M. S., R. S. Ettinger, P. Hermanns, B. Lee, G. Carlsson, M. Taskinen and O. Makitie, "The natural history of severe anemia in cartilage-hair hypoplasia," *Am J Med Genet A,* 138, 35-40 (2005).
Virolainen, M., E. Savilahti, I. Kaitila and J. Perheentupa, "Cellular and humoral immmunity in cartilage-hair hypoplasia," *Pediatr Res,* 12, 961-6 (1978).
Vlug, A., E. J. Nieuwenhuys, R. V. van Eijk, H. G. Geertzen and A. J. van Houte, "Nephelometric measurements of human IgG subclasses and their reference ranges," *Ann Biol Clin (Paris),* 52, 561-7 (1994).
Wolska-Kusnierz, B., H. Gregorek, K. Chrzanowska, B. Piatosa, B. Pietrucha, E. Heropolitanska-Pliszka, M. Pac, M. Klaudel-Dreszler, L. Kostyuchenko, S. Pasic, L. Marodi, B. H. Belohradsky, P. Ciznar, A. Shcherbina, S. S. Kilic, U. Baumann, M. G. Seidel, A. R. Gennery, M. Syczewska, B. Mikoluc, K. Kalwak, J. Styczynski, A. Pieczonka, K. Drabko, A. Wakulinska, B. Gathmann, M. H. Albert, U. Skarzynska and E. Bernatowska, "Nijmegen Breakage Syndrome: Clinical and Immunological Features, Long-Term Outcome and Treatment Options - a Retrospective Analysis," *J Clin Immunol,* 35, 538-49 (2015).
Vulliamy, T., A. Marrone, F. Goldman, A. Dearlove, M. Bessler, P. J. Mason and I. Dokal, "The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita," *Nature,* 413, 432-5 (2001).
Zhao, L., D. Dhall, Z. Cheng, H. L. Wang, T. M. Doherty, C. Bresee and P. K. Frykman, "Murine model of Hirschsprung-associated enterocolitis II: Surgical correction of aganglionosis does not eliminate enterocolitis," *J Pediatr Surg,* 45, 206-11; discussion 211-2 (2010).

12. ORIGINAL PUBLICATIONS