Diploma thesis

Immunological aspects of Constitutional Mismatch Repair- Deficiency Syndrome (CMMRD) in Pediatric Hematology-Oncology

submitted by

Victoria Katharina Tesch

to attain the academic degree

Doctor medicinae universae

(Dr. med. univ.)

at the

Medical University of Graz

conducted at the

Departement of Pediatric and Adolescent Medicine/ Division for Pediatric Hematology-Oncology

Under supervision of

Univ. Prof. Dr. Markus Seidel

Univ.Prof. Dr. Wolfgang Schwinger

Graz, June 29, 2017

Affirmation in lieu of an Oath

I hereby formally declare, that I have written the submitted thesis independently and without any illegitimate assistance from third parties. I confirm, that I used no other than the declared sources for the preparation of this academic work. All used sources have been indicated as such and acknowledged by means of complete references in the text.

Graz, June 29, 2017 *Victoria Katharina Tesch eh*

Für Opa.
In deine Fußstapfen treten und doch eigene Spuren hinterlassen.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to Professor Markus Seidel, who offered me this project I passionately worked on and made it possible in the beginning. With his immense motivation and knowledge, he managed to spark a passion for research on me. Thank you for guiding me greatly through the process of preparing this thesis.
I would also like to especially thank Prof. Dr. Katharina Wimmer for her time and help in the process of gathering data. Furthermore, I want to express my gratitude to Dr. Daniel Rueda-Fernandez, Dr. Chrystelle Colas, Dr. Laurence Brugieres, Prof. Dr. Michaela Nathrath, Dr. Hagit Baris Feldman and all other physicians from participating centers who helped to establish the collection of data.

Since this thesis symbolizes the end of my studies and an important and exciting chapter of my life, I want to thank those who went all the way with me.
My deepest gratitude goes to my whole family- most importantly my parents Renate and Peter, who with their great generosity were always lovingly there for me, encouraged me all the way long, inspired my curiosity and my interest in medicine and made my medical education possible. I would not have accomplished all this without you as my biggest supporters! Mama, thank you for your positivity, for soothing my perfectionism and for giving me emotional backup whenever I needed it. Papa, without you as a teacher in life I would truly not be who and where I am now. Thank you for showing me, with your unconditional love and help, that I am never alone in this.
Furthermore, I would like to thank my little brother Alexander, my future parents in law and my wonderful grandparents for going all the way with me. Opa, thank you for being my biggest rolemodel and inspiration for my future profession.

A big thank you goes out to all my friends, who with their company filled this chapter of life with laughs, a strong sense of togetherness and unforgettable memories.

Manuel, my companion for life. Thank you for standing by my side and loving and supporting me no matter what. You always showed me, that I can achieve anything- with your unconditional belief in me, whenever I lost mine.

Zusammenfassung

Hintergrund: Biallelische Mutationen in Mismatch Reperatur (MMR) Genen führen zu konstitutioneller Mismatch Reperatur Defizienz (CMMRD), einem erst in 198 PatientInnen beschriebenen äußerst seltenen Tumorneigungssyndrom mit erheblichem Risiko schon im Kindesalter Malignome zu entwickeln. Mismatch Reperatur Gene sind nicht nur wichtig um die genomische Stabilität aufrecht zu erhalten, sondern spielen auch eine Rolle im Klassenwechsel der Immunoglobuline (CSR) und der somatischen Hypermutation (SHM). Diese Prozesse sind in der B-Zellreifung und im Sinne der Erlangung eines vielfältigen Immunoglobulinrepertoires essentiell. Es wurde daher, wie erwartet, bereits gezeigt, dass CMMRD mit Defekten in CSR und SHM einhergeht, was wiederum zu klinisch relevanten Immundefekten führen könnte. Dieses Projekt beleuchtete sowohl den zellulären und humoralen in vivo Immunphänotyp, als auch den bisher relativ unerforschten klinischen Immunphänotyp von CMMRD PatientInnen aus Europa und dem mittleren Osten, um unser Verständnis für die Rolle von MMR in der Antikörperentwicklung zu vergrößern. Eine genaue klinische Beschreibung der PatientInnen, die an diesem seltenen Syndrom leiden, wurde ebenfalls angestrebt.

Methoden: Eine retrospektive und deskriptive Evaluierung einer PatientInnenserie, welche 11 CMMRD PatientInnen aus 8 verschiedenen Zentren umfasste, wurde im Rahmen dieser Studie durchgeführt. Teilnehmende Zentren ließen uns ausgefüllte klinische Fragebögen sowie Laborparameter zukommen. Die gesammelten Daten wurden anonymisiert, analysiert und hinsichtlich Immundefizienz interpretiert. Die ausführliche deskriptive Analyse von klinischen und immunologischen Merkmalen wurde mit Hilfe von Microsoft Excel© Version 2016 und Graph Pad Prism© durchgeführt.

Ergebnisse und Conclusio: 20 Malignome, darunter 7 hämatologische Malignome (35%), 6 maligne Hirntumore (30%), 5 Lynch-Syndrom assoziierte Malignome (25%), 1 Wilms Tumor (5%) und 1 maligner Phylloides Tumor (5%) wurden in dieser Fallserie in 10 von 11 PatientInnen (90,91%) diagnostiziert. Die meisten PatientInnen (n=6) trugen eine biallelische PMS2 Mutation (54,55%), drei PatientInnen trugen eine biallelische MSH6 Mutation (27,27%) und weitere zwei eine bialleische MLH1 Mutation (18,18%). Die Familienanamnese hinsichtlich Malignitäten war in neun PatientInnen negativ und elterliche Blutsverwandschaft war in sieben PatientInnen erhebbar. Alle inkludierten PatientInnen zeigten

nichtmaligne Merkmale, wie Cafe-au-lait-Flecken. Die in dieser Fallserie erhobenen klinischen Resultate bekräftigen frühere Beschreibungen der klinischen Präsentation dieses PatientInnenguts. Die Analyse hinsichtlich immunologischer Aspekte ergab keinen uniformen zellulären/humoralen Immunphänotyp in dieser PatientInnengruppe. Die vorhandenen Resultate beinhalten unter anderem verminderte klassengewechselte B-Gedächtniszellen in 5 von 10 PatientInnen (50%) und Immunglobulindefizienzen in allen bis auf 2 PatientInnen (80%) und lassen somit auf einen partiellen in vivo CSR Defekt schliessen. Die erwartete hohe IgM Konzentration konnte jedoch nur in 2 PatientInnen gezeigt werden (20%). Klinische Korellate, die in dieser Studie erstmals systematisch in CMMRD PatientInnen analysiert wurden, konnten einheitlich als unauffällig hinsichtlich Immundefekten interpretiert werden. Weitere prospektive Studien mit größerer Fallzahl werden von Nöten sein um die Evidenz des Wissenstandes dieses seltenen Syndromes, welches zu großen Teilen noch auf Beobachtungen basiert, sowohl in klinischer als auch in immunologischer Hinsicht, zu verbessern.

Abstract

Background: Biallelic mutations in Mismatch repair (MMR) genes result in a condition referred to as Constitutional Mismatch Repair- Deficiency Syndrome (CMMRD), a rare but devastating Cancer predisposition syndrome with a high chance of developing childhood malignancies, reported in a total of only 198 patients to date. The MMR system is, apart from its main function of maintaining genomic stability, involved in Class switch recombination (CSR) and somatic hypermutation (SHM). Both processes are needed for B cell maturation and diversity of our immunoglobuline repertoire. It is therefore expected and has already been shown that CMMRD may lead to defective CSR and SHM, which in turn might result in clinically relevant immunodeficiencies. This project addressed both the in vivo cellular/ humoral and the to date unexplored clinical immunophenotype of CMMRD patients across Europe and the Middle East to increase our understanding of the role of MMR in immunoglobuline maturation. A thourough clinical description of patients suffering from this rare condition, was furthermore approached.

Patients and Methods: A retrospective and descriptive evaluation of a patient series including 11 CMMRD patients from 8 different centers across Europe and the middle East was conducted. Participating centers provided us with filled out clinical questionnaires and laboratory parameters of the cellular and humoral immune system. Collected data were anonymized, analyzed and interpreted regarding immunodeficiency. The thourough descriptive analysis of clinical and immunological aspects was conducted using Microsoft Excel© Version 2016 and Graph Pad Prism©.

Results & Conclusion: 20 malignancies were diagnosed in 10 out of 11 patients (90,91%), 7 hematological malignancies (35%), 6 malignant brain tumours (30%), 5 Lynch-syndrome associated malignancies (25%), 1 Wilms tumour (5%) and 1 malignant Phylloides tumour (5%). Most patients (n=6) included carried a biallelic PMS2 mutation (54,55%), three patients carried a bialleic MSH6 mutation (27,27%) and another two patients were found to carry a biallleic MSH2 mutation (18,18%). Family history concerning cancer was negative in nine patients and parental consanguinity was identified in seven patients. Nonmalignant features such as Café au lait maculae were exhibited by all patients. Clinical findings of this case series therefore strengthen previous descriptions of clinical presentation. Analysis of

immunological aspects revealed no uniform cellular/humoral immunophenotype in this patient population. Partial in vivo CSR impairment is however implied by the results of our study including reduced circulating memory cells that have undergone class switch in 5 out of 10 patients (50%) and Immunoglobuline deficiencies in all but two patients (80%). The expected high levels of IgM were only detected in 2 patients (20%). Clinical correlates regarding immunodeficiencies, that were systematically analyzed in CMMRD patients for the first time in this study, were uniformly found not to be remarkable. Further prospective studies with larger patient numbers will be needed to add evidence to the knowledge, both clinical as well as immunological, of this rare syndrome, that is to date markedly based on observations.

Table of contents

Abbreviations

A	adenine
Ab	antibody
ACC	agenesis of the corpus callosum
AD	autosomal dominant
AID	activation induced cytidine deaminase
ALL	acute lymphatic leukemia
AML	acute myeloid leukemia
ANA	anti nuclear antibodies
APC	adenomatous polyposis coli gene
APE1	apurinic/apyrimidinic (AP) endonuclease
AR	autosomal recessive
ATM	ataxia-telangiectasia mutated,
B-NHL	B-cell Non-Hodgkin lymphoma
b2GP	beta 2 Glykoprotein
BCR	B-cell receptor
BER	base excision repair
BLM	Bloom syndrome RecQ like helicase
BM	bone marrow
BRCA1/BRCA2	breast cancer1/breast cancer2
C	cytosine
Ca	carcinoma
CALM	Café au lait macula
CD	cluster of differentiation
CDKN1C	cyclin-dependent kinase inhibitor 1C
CMV	cytomegalovirus
CPG	cancer predisposition gene
CPS	cancer predisposition syndrome
CRC	Colorectal cancer
csBm	Class switched B-memory cells
CSR	class switch recombination
CT	Computer tomography
CVID	Common variable immunodeficiency

DNA	deoxyribonucleic acid
DSB	double strand break
e.g.	exempli gratia, for example
EBV	Ebstein-Barr virus
ENT	ear-nose-throat tract
EXO1	Exonuclease1
f	female
Fab	Fragment antigen binding
FACS	Fluorescence- activated cell sorting
FANC	Fanconi anemia completation group
FAP	familiar adenomatous polyposis
G	guanine
g	gramm
GC	Germinal center
GI	gastrointestinal
HB	Hepatitis B
HBS-AB	Hepatitis B surface antibodies
HiB	Hemophilus influenzae B
HIGM	Hyper-IgM syndrome
HR	homologous recombination
i.e.	id est, that is
ICOS	inducible costimulator
IDL	insertion-deletion loops
Ig	immunoglobuline
Ig-CSR-Ds	Immunoglobulin class- switch recombination deficiencies
IgAD	Immunoglobuline A-deficiency
IGF 2	insulin-like growth factor 2
IHC	immunohistochemistry
L	litre
LN	Lymph node
m	male
MEN1	Multiple endocrine neoplasia type 1
MLH1	mutL homolog 1

MMR	mismatch repair
Mono	monocytes
MRI	Magnetic resonance imaging
MSH2	mutS homolog 2
MSH6	mutS homolog 6
MSI	microsatellite instability
MZ	Marginal zone
n.a.	Not answered, not available
n.d.	not defined
n.t.	not tested
NBN	nibrin
ncsBm	non-class switched B-memory cells
NER	nucleotide excision repair
NF1/NF2	Neurofibromatosis type 1/type2
NHEJ	non-homologous endjoining
NK	natural killer cell
PB	peripheral blood
PC	plasma cell
PCNA	proliferating-Cell-Nuclear-Antigen
PID	primary immunodeficiency
PMS2	PMS1 homolog 2
PPAP	polymerase proofreading associated polyposis
PTEN	phosphatase and tensin homolog
PTPN11	tyrosine-protein phosphatase non-receptor type 11
RAF 1	rapidly accelerating fibrosarcoma 1
RAG1, RAG2	recombination activating gene1,2
RB1	retinoblastoma 1
rec.	recessive
RET	receptor tyrosine kinase Ret
RFC	replication factor c
RNA	ribonucleic acid
RSS	recombination signal sequences
RUNX1	runt-related transcription factor 1
SCID	severe combined immunodeficiency

SD	standard deviation
SDHA	succinate dehydrogenase complex, subunit A
SHM	somatic hypermutation
SLE	systemic lupus erythematosus
T	thymine
T-NHL	T-cell Non-Hodgkin lymphoma
TACI	transmembrane activator and CAML interactor
TCR	T-cell receptor
TLS	translesion DNA synthesis
TP53	tumour protein p53
TSC1/TSC2	tuberous sclerosis 1/2
U	uracil
UNG	Uracil DNA glycosidase
VDJ gene segments	variable, diversity, joining gene segments
VZV	Varicella zoster virus
WBMRI	whole body magnetic resonance imaging
WRN	Werner syndrome ATP- dependent helicase
XP	Xeroderma pigmentosum
XR	X-linked recessive
y	year(s)

List of figures

List of tables

1. Introduction

1.1 Hereditary Cancer predisposition

Genetics and heredity play an important role in the multifactorial etiology of cancer. Even though 90%, and therefore the vast majority of all cancers diagnosed are caused by so called „de novo“ somatic mutations,the remaining and not insignificant 10 % are caused by mutations, that are inherited (1). There are several genetic factors that are known to influence the lifetime risk of developing malignancies. When we talk about the factors involved in cancer progression, many of the mutations causing malignancies particularly affect DNA repair, cell cycle regulation and cell-death pathways. Like mentioned above, mutations in those specific loci can either be somatic or inherited, with the latter being a germline mutation (1-3). This results in the carrier of a germline mutation being susceptible to a certain spectrum of cancers, depending on which class of genes is affected. Tumour suppresor genes, oncogenes, DNA repair genes and angiogenesis genes represent the four most important classes (4). Cancer predisposition genes (CPG) mostly act as tumour suppressors and can predispose to cancer through loss of their function. Gain-of- function mutations represent the minority and include genes that encode for kinases like MET, ALK and KIT (1, 5). Over the last thirty years, a total of 114 cancer predisposition genes (CPG), in which relevant mutations lead to either high or moderate cancer risk, meaning a resulting $\geq$2 fold relative risk, or at least 5 % of mutation carriers developing cancer, were discovered, with the majority of these genes albeit being associated with a considerably higher risk and penetrance (6). Resulting Cancer Predisposition Syndromes (CPS) can be inherited in an autosomal dominant, autosomal recessive, X-linked recessive or – as can be seen in only one case -Y-linked way (1, 6). A lot of known CPS show an autosomal dominant pattern of inheritance, in which a heterozygous germline mutation predisposes towards early-onset malignancies. Rarely occuring are biallelic mutations affecting CPS genes, that show an autosomal recessive pattern and cause a similiar phenotype, but cancer can develop at an even earlier age. Although the specific genetic mutations have been discovered and described for a lot of CPS, there are to date not as many clear descriptions of the correlation between this genotype and the resulting phenotype (7). Predisposition for cancer itself albeit has long been

recognized, with the first description of it being a publication authored in 1866 by the neuroanatomist Paul Broca, where he described 15 cases of breast cancer in his wife's family, proposing this to be the evidence for hereditary predispositon towards developing malignancies (6, 8). In 1987,121 years later, the first cancer predisposition gene, the retinoblastoma predisposition gene- *RB1*, was discovered. Knudson´s suggestion of a „two hit model ", with one allele being mutated in the germline and one allele being mutated somatically, therefore an inactivation of both alleles necessary in order to develop cancer is given, turned out to be true (6, 9).

1.1.1 Cancer predisposition syndromes (CPS)

A variety of syndromes that hereditarily predispose an individual to cancer are known (1). The 114 known cancer predisposition genes (CPG) mentioned above (6) in which mutations results in cancer predisposition syndromes (CPS) show different patterns of inheritance (1, 6). 65 CPG show an autosomal dominant inheritance pattern, 28 show an autosomal recessive inheritance pattern, four show an X-linked inheritance pattern and one syndrome shows a Y-linked inheritance pattern. 16 of the mentioned CPG can lead to both autosomal dominant and autosomal recessive conditions, meaning that either monoallelic or biallelic mutation carriers show symptoms of the resulting CPS (6).

Some CPS, such as Beckwith –Wiedemann Syndrome, only predipsose children to cancer, but for the majority of CPS increased risk for developing malignancies begins in childhood and continues in their adult life (10), whereas other syndromes mainly affect adults (6).

Amongst other things, adult type cancers occuring in children are a hint given in the direction of a CPS (11).

1.1.1.1 X linked CPS

Despite the fact that the majority of CPS do not follow a pattern of X-linked recessive inheritance (6), some important examples should be mentioned.

<u>Simpson Golabi Behmel Syndrome</u>

A mutation in the gene *GPC3* mostly leads to predisposition for Wilm´s tumor and hepatoblastoma (1).

<u>X-linked lymphoproliferative disease</u>

A mutation in the gene *SH2D1A* leads to a predisposition for Lymphoma (1).

1.1.1.2 Autosomal dominant CPS

As mentioned above 65 CPG- the majority -show an autosomal dominant inheritance pattern. The following list is not intended to be exhaustive, but should rather show some examples of CPS that lead to phenotypes in monoallelic mutation carriers.

Adenomatosis polyposis, familial

A mutation in the gene *APC* can lead to malignancies mainly located in the colon (1).

Beckwith-Wiedemann Syndrome

Mutations in *IGF-2* or *CDKN1C*, among others, can lead to a variety of malignancies including Wilms tumor and hepatoblastoma (1, 10).

Canale-Smith Syndrome

A mutation in the gene *FAS* leads to a prediposition for lymphomas (1).

Cowden Syndrome

A *PTEN* mutation leads to a predisposition for multipe types of malignancies (1).

Dicer 1 syndrome

A mutated gene called *DICER1* leads to an increased risk for developing pleuropulmonary blastoma (1).

GATA2 haploinsufficiency syndrome

A *GATA2* mutation results in predisposition for Acute myelotic leukemia (AML) in monoallelic mutation carriers (1).

Hereditary breast-ovarian cancer syndrome

Mutations in *BRCA1* or *BRCA2* result in the well known predisposition for breast and ovarian cancer in adults (1).

Leopard Syndrome

PTPN11/RAF1 mutations can result in AML (1).

Li- Fraumeni Syndrome

Multiple types of malignancies, including leukemia (11), can result from a mutation in *TP53* (1).

Lynch Syndrome

Mutations in *MLH1, MSH2, MSH6* and *PMS2* can lead to colorectal cancer and other malignancies predominantly seen in adults (1).

Multiple endocrine neoplasia type 1/2

MEN1 or *RET* mutations predispose for malignancies situated in the endocrine glands (1, 10).

Neurofibromatosis type 1/2

The main tumor sites in *NF1/2* monoallelic mutation carriers are in th brain and spine (1).

Retinoblastoma

The first discovered CPG, *RB1* (6, 9), predisposes for Retinoblastoma (1) and Osteosarcoma (10).

Severe congenital neutropenia

A mutation in the gene *RUNX1* predisposes for AML (1).

Tuberous sclerosis

A *TSC1/2* mutation results in a high risk for developing, among others, astrocytomas (1, 10-13).

1.1.1.3 Autosomal recessive CPS

Half of the conditions, in which a phenotype is present in either monoallelic (AD) or biallelic (AR) mutation carriers, show a more severe form of the condition in biallelic mutation carriers. For example *BRCA2* mutation or *MLH1, MSH2, MSH6, PMS2* mutations in biallelic carriers predominantly predispose for childhood cancer whereas the same mutations in monoallelic carriers result in a higher risk for developing cancer as an adult (6). The other half of this group of genes, e.g. *SDHA*, leads to cancer in monoallelic carriers but has no reported cases of cancer in biallelic carriers. However, that is very likely caused by early mortality of other symptoms of the resulting phenotype.

Ataxia teleangiectasia

A mutation in the gene *ATM* predisposes for lymphomas and leukemia (1).

Bloom syndrome

BLM mutation can result in multiple types of cancer (1) including leukemia and lymphomas (10).

Fanconi anemia

Mutated genes of the *FANC* family mainly predispose for AML (1), but also lead to a variety of other malignancies (10).

Nijmegen breakage syndrome

A *NBN* mutation (1) predisposes biallelic carriers for lymphoma, medulloblastoma, gliomas and rhabdomyosarcomas (10).

Werner syndrome

A biallelic *WRN* mutation results in a higher risk for developing, among other things, melanomas and sarcomas (1, 10).

Xeroderma pigmentosum

Mutations in one of the genes out of the *XP* family mainly leads to malignancies of the skin (1).

Constitutional mismatch repair deficiency (CMMRD)

Biallelic mutation in one of the mismatch repair genes *MLH1, MSH2, MSH6, PMS2* predisposes for different childhood malignancies (14), that will be discussed extensively in the following chapters of this thesis.

1.1.1.4 Hematologically/ Immunologically relevant CPS

Roughly 60 genes to date have been discovered, that increase the risk for developing hematological malignancies (11). The following table, **Table 1**, shows an overview of CPS that are especially relevant in hematology and immunology. The table does not by any means claim to be complete. Underlined syndromes show defects in DNA repair pathways (1, 10, 11, 13-16).

Table 1: Hematologically/Immunologically relevant CPS (1, 11)

XR	AR	AD
X-linked lymphoproliferative disease	Ataxia teleangiectasia	Canale Smith syndrome
	Bloom syndrome	GATA2 haploinsufficiency syndrome
	Fanconi anemia	Leopard Syndrome
	Nijmegen breakage syndrome	Severe congenital neutropenia
	Constitutional mismatch repair deficiency syndrome (CMMRD)	Neurofibromatosis Type 1
		Li-Fraumeni syndrome

A lot of different mechanisms can lead to CPS predisposing for hematological malignancies. Six main groups, however, can be divided: Genetic instability/DNA repair syndromes (e.g. underlined syndroms in **Table 1**), cell cycle/differentiation

(e.g. Neurofibromatosis type 1), bone marrow failure syndromes (e.g. severe congenital neutropenia), telomere maintenance, immunodeficiency syndromes (e.g. Wiskott –Aldrich), transcription factors/pure familial leukemia syndromes (11, 15, 17). A considerably high number of CPS, with particular emphasis on Hematology, as shown in **Table 1**, are caused by defective DNA repair (11).

1.1.1.5 DNA repair mechanisms in hereditary cancer predisposition

We constantly ought to deal with exposure to DNA damage, may it be endogenous processes such as meiosis or DNA replication or exogenous agents like ionising radiation. In order to ensure mantaining of genomic stability and to prevent mutagenesis, certain pathways have evolved to deal with the arising damage and defects (18). Genomic instability and a higher mutation rate are in fact main features of cancer cells and are precisely caused by faulty DNA repair (19, 20). A higher tolerance for DNA damage can either result from losing the system that normally signals it or from defects in one of the DNA repair pathways itself. Six main DNA repair pathways are known: Mismatch repair (MMR), Base excision repair (BER), Nucleotide excision repair (NER), Homologous recombination (HR), Nonhomologous endjoining (NHEJ), Translesion DNA synthesis (TLS) (15, 20). Any of these pathways can be affected, which implies a higher rate of chromosome breakage and mutations, and thus result in a phenotype, that differs in terms of detected mutation, drug sensitivity and treatment outcome depending on the respectively affected pathway (20).

1.2 The Mismatch repair system

1.2.1.1 DNA Mismatch repair

The DNA mismatch repair system (MMR), first described in the 1960s in fungi and bacteria (21), plays a key role in mantaining genomic stability and integrity. Its main function is the detection and elimination of non Watson-Crick base pairs (22, 23) and strand misalignements, i.e. insertion-deletion loops (IDL), that arise during DNA replication (21, 24-26). Holding such abilities enables the MMR system to increase replication fidelity by up to three orders of magnitude (26).

Mismatches in base pairing epitomize a special type of „DNA damage“ as they only exist for a certain amount of time and do not contain damaged DNA at all (21).

Errors occuring during DNA replication, for example an A-C mismatch, only exist when the two strands stay annealed. As soon as the two strands separate there is no damage in need of repair noteable and unrepaired errors thus are established in 50 percent of the progeny DNA. Consequntly the **first rule** for efficient mismatch repair states that is has to occur before the next round of DNA replication (21). Permanent mutations in cells are therefore averted by the MMR system (24).

Insertion deletion loops, named as such because they lead to insertions or deletions in progeny DNA if they remain unrepaired, exemplify a distinct condition.

Slippage of the primer strand in a repetitive fragment of DNA, such as microsatellites consisting of mono, di or trinucleotide repeats, results in IDL. If the aforesaid situation occurs behind the polymerase, whose duty is to proofread, the mistake will not be detected by the latter and therefore reperation relies on the MMR System alone (21, 27). Microsatellites, short repeat units, are especially affected by slippage. Cells that are missing the MMR system predominantly show instability in microsatellites, which is known as microsatellite instabilty (MSI) (21, 25, 28). In order to accomplish the task of efficient MMR the **second rule** reads as follows: The MMR system, with its recognition factors, needs to recognize all types of base mismatches and IDLs, that can show various structures (21). When recognizing a mismatch, another issue occurs: the MMR system needs to know which of the both strands is the one with the correct genetic information and which is the one containing the error. According to the definiton that the template strand always carries the correct genetic information, the MMR system therefore needs to be directed to the nascent DNA strand carrying the mutation (21). This also articulates the **third rule** for efficient MMR (21). The MMR system must be capable of distinguishing between the template and the nascent strand in order to repair the nascent strand, as it is the one conatining the genetic error (21, 26).

The process of repairing replication errors consists of three main tasks.

1. Mismatch recognition
2. Excision of the error in the nascent strand
3. Error free resynthesis

Several Mismatch Repair genes, first discovered in Escherichia coli (26), encode for proteins, that are required to carry out all the three main steps of repairing replication errors mentioned above (25, 26). (See **Table 2**)

MutS and MutL proteins are, amongst others, involved in this whole process.

Mismatch repair in humans starts with mismatch recognition, performed by one of the two Mut S heterodimers. MutS homologs (MSHs) in eukaryotes, distinguishing them from the ones discovered in bacteria, function as heterodimers but only MSH1,6,7,8 out of the 8 discovered to date have the ability to recognize mismatches (21). MutSα,the more plentiful heterodimer, consists of the two homologs and crucial MMR proteins MSH2 and MSH6, and is specialized in recognizing base/base mismatches and single nucleotide misalignements (21, 27). MutSβ, composed of MSH2 and MSH3, indispensable MMR proteins, merely recognizes larger IDLs. Either MutSα or MutSβ subsequently recruit a heterodimer called MutLα, that is made up of the proteins MLH1 and PMS2, referred to as MutL homologs (21, 26, 27). In humans, as opposed to the findings in Escherichia coli (21), MutLα has endonuclease activity, with the PMS2 unit submitting the process of introducing nicks in the region surrounding the mismatch in need of repair (29).

MutLα is also in charge of the interplay between the mismatch recognition complex and the other proteins needed (25), therefore it is commonly called the molecular matchmaker (21). For the next step an additional protein, namely exonuclease 1 (25), is needed. It is loaded at the 5´side of the mismatch, which then activates the 5´-3´ exonuclease in charge of removing the part oft he DNA carrying the errogenous genetic information (26). The resulting gap, which is reasonably single stranded, is filled by DNA Polymerase δ, an additional enzyme, and its cofactors proliferating cell nuclear antigen (PCNA) and replication factor C (RFC). At last and in order to complete the complex process of replication repair, the residual nick has to be ligated by DNA ligase 1 (26, 27, 30).

Table 2 summarizes the most important eucaryotic MMR factors involved in the process and their functions:

Table 2: MMR factors and functions

Eukaryotic factor	Function
MutSα (MSH2-MSH6)	Mismatch recognition Repairs: Single base-base mismatches, small IDLs
Mutsβ (MSH2-MSH3)	Mismatch recognition Repairs: larger IDLs
MutLα (MLH1-PMS2)	Molecular matchmaker coordinating events, strand specific endonuclease function(29), terminates excision
MutLβ (MLH1-MLH2), MutLγ (MLH1-MLH3)	Exact function of human heterodimers unknown
RFC complex	PCNA loading, activates MutLα endonuclease
PCNA	Interaction with Muts and MutL homologs, molecular matchmaker, recruits MMR proteins to mismatches, repair synthesis
EXO1	5´- 3´ Exonuclease, excision of dsDNA
DNA Pol δ	Repair synthesis
RPA	Participation in excision and DNA synthesis, single stranded DNA binding protein
HMGB1	Accessory protein, stimulates excision
PARP	Accessory protein, improves mismatch selectivity
DNA ligase	Sealing nicks after DNS synthesis, completes repair process

modified from tables in Jiricny et al. 2013 (21) & Kunkel and Erie 2005 (24)

1.2.2 Other functions of the MMR System

The MMR System is known to not only play a role in the complex mechanism of DNA mismatch repair itself, but also a variety of other pathways of DNA metabolism (21).

DNA Recombination, homologous recombination and nonhomologous end joining of double strand breaks are some of the processes that were found to involve the MMR Proteins and were therefore intensely reviewed (21, 31).

Interestingly, MMR proteins also play a role in the apoptotic response to DNA damage. It was discovered that cells that are MMR deficient are tremendously more resistant to apoptosis and therefore more prone to tolerant DNA damage induced by O^6 – Methylguanin, 6-Thioguanin or Cisplatin. The exact mechanisms, however,

are not sufficiently understood yet. The common opinion today is, that MMR leads to cell-cycle arrest after noticing several tries of insufficient DNA repair after exposure to DNA damage. There is also evidence for MMR to directly signal apoptosis (26, 27, 32).

MMR proteins also have functions in dealing with oxidative DNA damage (21, 33), repair of interstrand cross links and antibody diversification (21), more precisely class switch recombination (CSR) and somatic hypermutation (SHM) (34), which will be dicussed in following chapters. However, additional functions of MMR still need to be understood better (21, 27, 32).

1.2.3 Defective Mismatch repair and Cancer/ Lynch Syndrome

Defects concerning the MMR system lead to a decrease in genomic integrity and an increase in resistance to genotoxic agents and not to forget, microsatellite instabilty (MSI), caused by lack of repair in small IDL, which in turn results in accumulation of errors in those repetitive sequences (21, 25, 35). It is therefore by no means surprising and known for over two decades (26), that failure in accomplishing the important functions of the MMR system, especially mainting genomic stabilty, may lead to cancer (25). Monoallelic germline mutations in one of the four MMR genes *MLH1, MSH2, MSH6, PMS2*, with their functions discussed above, lead to Lynch syndrome (LS), showing an autosomal dominant pattern of inheritance and mainly predisposing for malignancies located in the colon or the endometrium, as well as the ovaries, the urinary tract, the stomach, the small bowel and the brain.The onset is in adulthood, but still a lot earlier than in the general population (14, 21, 26, 36-39). *MSH2* and *MLH1* are the most common mutations found in Lynch syndrome, also known as hereditary nonpolyposis colon cancer (HNPCC). The development of malignancies in LS patients, commonly not before the forth decade of life, is the consequence of somatic loss of the reamining wild type MMR gene which results in impaired mismatch repair, which in turn leads to MSI, used for detecting the MMR defect in the resulting tumours (21, 26, 40). It is only known more recently that also biallelic germline mutations with an autosomale recessive pattern of inheritance in the MMR genes rarely occur. The resulting condition lacks adequate DNA MMR activity completely from birth, thus differs from Lynch syndrome, where sufficient MMR function is present until the wild type allele is inactivated, and is referred to as Constitutional mismatch repair-deficiency syndrome (CMMRD) (14, 41, 42).

1.3 Constitutional Mismatch Repair-Deficiency Syndrome (CMMRD)

Biallelic homozygous or compound heterozygous deleterious mutations in one of the four MMR genes are the underlying genetic cause of CMMRD, a rare but well pronounced and devastating CPS (26, 36, 43, 44). The syndrome was first picked up on in 1999 when Ricciardone et al. (45) and Wang et al. (46) published characteristic case reports describing the phenotype of five children from consanguineous marriages within HNPCC families, who all carried a homozygous *MLH1* germline mutation, at the same time (26, 36, 39, 45, 46). However, in 1959 Jaques Turcot probably already described CMMRD patients, when he reported on siblings with colorectal polyps, colorectal carcinoma and primary malignancies within the brain (47). From that time on all children with colorectal malignancies and brain tumours were considered to suffer from the so called Turcot syndrome, albeit retrospectively viewed they were very likely CMMRD patients (36).

The name of the condition- Constitutional mismatch repair deficiency syndrome- itself, is very descriptive. Mismatch repair deficiency - explaining and recapitulating the defect, and constitutional, stating it to be a germline mutation, which underlines the hereditary aspect of the condition in order to not confuse it with somatic MMR mutations in Lynch syndrome associated tumours or sporadically occuring MMR deficiency in colorectal cancer patients (26). However, other names like biallelic mismatch repair deficiency syndrom (BMMRD) (41, 48), childhood cancer syndrome (CCS) (49), CoLoN (Colon tumours and/or Leukemia/Lymphoma and/or Neurofibromatosis features) (50) or simply Mismatch repair deficiency syndrome(MMRD) (51), have also been used (26).

1.3.1 Epidemiology

Numbers of prevalence, incidence and risks for CMMRD can only be estimated (see Rana and Syngal 2017 (48)). Publications, however, suppose that the incidence of this exceedingly rare and commonly unrecognized CPS might be underestimated (26, 35) as a study conducted in Sweden by Magnusson et.al. in 2008 (52) revealed, that the risk of childhood malignancies in families with LS is remarkably increased (OR 29%) compared to control families without LS (26, 52). In support of this contention, also LS prevalence was highly underestimated as it was recently found to be 1/279 and therefore a lot higher than previously thought (48, 53).

1.3.2 History of CMMRD patients

1.3.2.1 Family history

One would suspect patients carrying a biallelic germline mutation in one of the MMR genes to already raise suspicion by having an indicative family history concerning cancer (40). In fact, the opposite is the case, as the family history of cancers mostly is inconspicuous and does thererfore not contribute a lot (41). Parents, posssibly both, of CMMRD patients carry a monoallelic mutation in a MMR gene (14, 43) and still, LS-related cancer history is often absent in CMMRD pedigrees (41), according to what Ripperger et al. reviewed 2010 in up to 85% (43). Parents are often too young to develop malignancies associated with the monoallelic MMR mutation they harbour and furthermore AR diseases in small families give the impression to be sporadic (42). However, interestingly, especially CMMRD patients carrying a PMS2 mutation often lack a clear family history of LS. An explanation for that is, that monoallelic *MLH1* and *MSH2* mutation show a higher penetrance of Lynch Syndrome associated malignancies than monoallelic *MSH6* (54) and particularly *PMS2* (55-57) mutations (26, 39).

However, family history regarding parental consanguinity in return is considerably more rewarding, as more than 50% of offsprings affected have parents that are blood related (14, 41, 58).

As siblings of CMMRD patients have a 25% risk to also suffer from the same condition clinicians should also look out for cancer history in those to further point them into the direction of a CPS (36). 50% of the children and the siblings of monoallelic MMR gene mutation carriers, easier said the parents, are at risk to carry a monoallelic mutation as well and may therfore suffer from LS. So this rare syndrome might have wide reaching consequences by involving a whole family (43).

1.3.3 Mutations in CMMRD

In comparison to LS, where *MLH1* and *MSH2* mutations make up for the majority of mutations (75%), it is only found in less than 20% of CMMRD patients (39). Out of the four MMR genes the mutation that is detected the most in CMMRD is a biallelic *PMS2* mutation (60% of patients), whereas frequency of biallelic mutations in the other MMR genes is distributed almost equally (36). In return, monoallelic *PMS2* mutations are only rarely seen in LS patients (5-15%) (39, 59), although a study of Truninger et al. (56) conducted in 2005 revealed, that among unselected colorectal

cancers monoallelic *PMS2* mutation are as commonly found as *MLH1/MSH2* mutations (26, 56). Still, there is an obvious predominance of *PMS2* mutations diagnosed in CMMRD.This might, amongst other things, be caused by a supposedly higher mutation frequency of *PMS2*, compared to the other MMR genes, in the population (26, 53). Furthermore some *MLH1* and *MSH2* mutations, who are rarely found in CMMRD patients, might be incompatible with life in a homozygous state (26), like, for example, some *BRCA2* mutations in breast cancer patients were found to be not viable when they occured biallelic (26, 60).This is supposed to be more improbable for *PMS2* mutations (39). In addition, CMMRD patients can harbour a variety of different germline mutations (e.g. truncating mutations, duplication mutations, missense mutations) (7).

1.3.4 Clinical features in CMMRD patients

As stated by Wimmer et al. (39) in 2016, almost 200 patients (198 patients out of 128 families) diagnosed with CMMRD, on grounds of a detected biallelic inactivating germline mutation in one of the four MMR genes, were reported to date (39). Description of clinical features therefore predominantly relies on publications, which are mainly individual case reports. Consequently selection/publication bias must always be kept in mind (36).

1.3.4.1 Malignancies in CMMRD patients

While Lynch syndrome patients basically don´t develop malignancies before reaching the age of 25, CMMRD represents a distinct childhood cancer syndrome (39), underlined by the fact that all the patients known so far developed at least one malignancy in their childhood and adolescence (39), and up to 40% even develop multiple primary cancers (61). The real cancer risk associated with CMMRD is not identified to date, but based on our current knowledge it is suggested to be exceedingly high (36).

The wide range of malignancies occuring in CMMRD patients can broadly be split into four groups, namely haematological malignancies, brain tumours, Lynch-Syndrom associated tumours and others (26, 36). Within the first group Non-Hodgkin Lymphomas, more specifically T-cell non-Hodgkins Lymphomas (T-NHL) (43), occur most frequently. The second group, composed of malignant brain tumours, shows a predominance of high grade Gliomas (62), which in addition represents the malignancy found in most CMMRD patients (40,6%) so far (7, 39,

41). Out of the LS-associated tumours (63), the colorectal carcinoma (CRC) develops most frequently (36, 39). Most of the malignancies that develop in CMMRD patients belong to the first three groups mentioned, but as the variety of malignancies in the fourth group, including sarcomas and embryonic tumours (36, 64), demonstrates (e.g. neuroblastoma, rhabdomyosarcoma, basal cell carcinoma, muco-epidermoid carcinoma of the parotis) practically any malignancy occuring in a patient carrying a biallelic germline mutation in one of the MMR genes can be CMMRD related (36, 39).

Hematological malignancies, especially T-NHL, typically occur in infants and preschoolers with a mean age of 6 years at diagnosis. Brain malignancies develop a bit later as the mean age of 9 years at diagnosis shows. LS-associated tumours establish in adolescents and therefore the latest with a mean age of 17 years at diagnosis (36, 39), which still is 30 years prior to the mean age of onset observable in LS (65, 66). The type of malignancy and therefore the respective age of onset depends on the mutation the patient harbours. *MLH1/MSH2* mutation carriers tend to develop hematological malignancies which are more apt to occur at an earlier age, than those carrying a *MSH6/PMS2* mutation, which seems to predispose more towards brain tumours and LS-associated tumours with a later onset (26, 36, 39). **Table 3** shows the genotype/phenotype correlation of different mutation carriers.

Table 3: Genotype/Phenotype correlation in CMMRD (14, 26, 36, 39)

	MLH1/MSH2	*MSH6/**PMS2***
mean age of malignancy onset	3,5 years	9 years
type of malignancy	hematological maligancies	brain tumours, LS-associated tumours
number of malignancies	more likely 1	more likely ≥ 1

PMS2 mutation carriers are found to survive their first malignancy more often and consequently tend to develop more than one malignancy in their childhood and adolescence, which supports the common opinion of *MLH1/MSH2* mutations resulting in the „more severe phenotype" of CMMRD, which again matches the findings of lower penetrance of *MSH6/PMS2* mutations in LS (26, 36, 39).

1.3.4.2 Premalignant and nonmalignant features in CMMRD patients

Apart from developing a broad range of malignancies early and frequent in life, there are a handful of typical nonmalignant or premalignant features found in patients suffering from CMMRD. The primarily diagnosed premalignancies are colorectal adenomas and polyps, which can be evocative of familiar adenomatous polyposis (FAP) when found in numbers up to 100 (36). The transformation into carcinomas, however, is unfortunately found to be extremely rapid in CMMRD patients (39, 63, 67). The acquisition of a somatic mutation in the polymerase proofreading genes polymerase δ and ε in the tumour in combination with the existing DNA MMR defect which in turn results in an ultrahypermutated tumour with unexampled progression might be the explanation for the rapid transformation (42, 68). Besides other gastrointestinal polyps, neurofibromas, that overlap with Neurofibromatosis type 1, and optical giomas that can develop, there is a nonmalignant tumour of the hair follicle, rarely found in healthy people, that should arouse suspicion, especially if present multiple times, in a child suffering from cancer. What is referred to by this, is the so called Pilomatricoma, also known under calcifying epithelioma of Malherbe (36). However, the most frequent, non- neoplastic finding observed in patients with CMMRD are hyperpigmented skin areas, amongst others the so-called Cafe´ au lait maculae (CALM), immediately reminiscent of Neurofibromatosis type 1 (NF1). The hyperpigmentated spots albeit often appear different than the classic ones, with uniform pigmentation and regular boarders, found in NF1 (36). CALMs in CMMRD patients thus show pigmentation to a different extent with areas of hypopigmentation, in addition the shape is said to be different, the borders more irregular and the lesion itself might as well be hairy (36, 41, 51). Some CMMRD patients, howbeit, show multiple classical NF1 CALMs, even if the number needed for diagnosis of NF1 (at least 6 CALMs) is oftentimes not reached. It rarely ever happens that a CMMRD patient has no areas of hyperpigmentation whatsoever but it ought to be kept in mind that the presence of one CALM in an individual is very frequent in the general population. Other signs reminiscent of NF1 (e.g. freckling, Lisch nodules, tibial pseudoarthrosis) as well as hypopigmentation (e.g. ash leaf spots) are also reported to occur in CMMRD patients and raise suspicion when found in a child with cancer (36).

Furthermore, there are some congenital malformations that, in comparison to the general population, are seen more often in CMMRD patients. Agenesis of the corpus

callosum (ACC) with or without grey matter heterotopia and non- therapy induced brain cavernomas represent the two most frequently found malformations (36, 39, 69). All in all, those premalignant and nonmalignant features, with a focus on skin abnormalities, add up to the clinal presentation of children with cancer carrying a biallelic germline mutation in one of the MMR genes (36).

1.3.5 Diagnosis of CMMRD

1.3.5.1 Differentials

As described above, there are a few conditions that overlap with CMMRD and might lead to a misdiagnosis or delayed CMMRD diagnosis as this syndrome is still often unrecognized by clinicians (35).

Features reminding clinicians of NF1, an autosomal dominant disorder (51), often lead to a false NF1 diagnosis even if the criteria for that are rarely ever met, the pattern of inheritance differs and germline NF1 mutations are usually not detectable (41, 51). However, there still is a possibility for CMMRD patients to carry a postzygotic NF1 mutation which might as well be present as a mosaic mutation (39, 70). The performance of an unsuitable NF1 surveillance protocol following a wrong NF1 diagnosis and the resulting delay of the correct CMMRD diagnosis might lead to missed malignancies associated with the latter (41).

Other conditions that can overlap with CMMRD in terms of their phenotype include Li-Fraumeni Syndrome (35), which predisposes for many early onset cancers including sarcomas (11), Polymerase proofreading-associated polyposis (PPAP) presenting with polyposis, adolescent onset of colorectal cancer, CALMs and pilomatricomas (71), FAP mainly presenting with colorectal adenomas and polyps which nearly all CMMRD patients who reach adolescence develop (36, 71) or other cancer susceptibility syndromes that are caused by constitutional DNA repair defects like MUTYH associated polyposis,where skin abnormalities can be found, or NTHL1 associated polyposis (71). Skin abnormalities in combination with cancer, more specifically AML and/or medulloblastoma are features occuring in chromosome breakage syndromes such as Fanconi anemia or Bloom syndrome but can be distinguished from CMMRD because of associated clinical features such as growth delay,microcephaly that are not found in CMMRD (51).

1.3.5.2 Diagnostic strategies

In a syndrome as rare as CMMRD with numerous overlaps and differentials, an often lacking family history and a clinical description that is solely based on the description of 198 known patients (39), it appears legitimate that clinicians frequently miss the hints and therefore fail to consider this diagnosis in a child with cancer (35). Thus, diagnosis of CMMRD with all its pitfalls constitutes a major challenge, genotypical as well as phenotypical (42).

The predominance of *PMS2* mutations in CMMRD patients has already been subject of discussion, but should be picked up in this chapter again. We already know that the genotype /phenotype correlation seems to vary between the different mutations. *PMS2* mutation carriers thus tend to survive longer and are therefore more likely to develop multiple malignancies with a predominance of brain tumours and colorectal malignancies. This resembles the phenotype Turcot described in 1959. Consequently Wimmer et al. (39) suggest that patients carrying a *PMS2* mutation might be picked up easier than *MLH1/MSH2* patients who often die at a very young age from hematological malignancies that in addition don´t raise suspicion in early childhood (7, 26, 39). This could thus also add up as an explanation for why *PMS2* mutations are as prevalent in CMMRD patients- they might simply be detected more frequently (39).

Even if patients with biallelic *PMS2* mutations are supposedly easier to recognize, the need for guidance remains and therefore in 2014 Wimmer et al. (36) proposed Diagnostic criteria based on the clinical phenotype consisting of a three- point scoring system to facilitate the diagnosis of CMMRD, as demonstrated in **Figure 1**. By using this indication criteria clinicians, that are often unfamiliar with this condition, should know when to raise suspicion in a child with cancer (36).

Indication for CMMRD testing in a cancer patient	≥3 points
Malignancies/premalignancies: one is mandatory; if more than one is present in the patient, add the points	
Carcinoma from the LS spectrum* at age <25 years	3 points
Multiple bowel adenomas at age <25 years and absence of *APC*/*MUTYH* mutation(s) or a single high-grade dysplasia adenoma at age <25 years	3 points
WHO grade III or IV glioma at age <25 years	2 points
NHL of T-cell lineage or sPNET at age <18 years	2 points
Any malignancy at age <18 years	1 point
Additional features: optional; if more than one of the following is present, add the points	
Clinical sign of NF1 and/or ≥2 hyperpigmented and/or hypopigmented skin alterations Ø>1 cm in the patient	2 points
Diagnosis of LS in a first-degree or second-degree relative	2 points
Carcinoma from LS spectrum* before the age of 60 in first-degree, second-degree, and third-degree relative	1 point
A sibling with carcinoma from the LS spectrum*, high-grade glioma, sPNET or NHL	2 points
A sibling with any type of childhood malignancy	1 point
Multiple pilomatricomas in the patient	2 points
One pilomatricoma in the patient	1 point
Agenesis of the corpus callosum or non-therapy-induced cavernoma in the patient	1 point
Consanguineous parents	1 point
Deficiency/reduced levels of IgG2/4 and/or IgA	1 point

*Colorectal, endometrial, small bowel, ureter, renal pelvis, biliary tract, stomach, bladder carcinoma.
CMMRD, constitutional mismatch repair deficiency; LS, Lynch syndrome; NHL, non-Hodgkin's lymphomas; sPNET, supratentorial primitive neuroectodermal tumours.

***Figure 1: Indication for testing in CMMRD**, taken from Wimmer et al. 2014 (36)*

A minimum of 3 reached points implicates an indication for testing with the two main methods widely used for screening being Immunohistochemistry (IHC) and MSI (7, 36).

IHC studies conducted by Baris et al. in 2015 (38) demonstrate the complete absence of functional protein in normal as well as tumour tissue caused by the involvement of both gene copies in CMMRD (38). In 2014 Bakry et al. (7) showed a specifity and sensitivity of 100 % for the detection of the mutated MMR gene using IHC in his cohort consisting of 26 tumours. Thus, they propose the possibility of using normal colonic or skin biopsies as fast guidance for genetic testing, which of course can not be replaced (7). However, it is said that non-truncating mutations like missense mutations might not be detected with this method that otherwise seems to be a helpful clinical device (7, 39, 72).

MSI analysis, first described in 1993 (73, 74), is a method used for detection of impaired MMR in mutation carriers in which it has a sensitivity of 93%. MSI, recognized by an increase of variation in length of short repetitive sequences, is a typical sign of non-functioning MMR and therefore analysis of it represents the Gold standard diagnostic method to detect this defect in LS associated malignancies even if the MMR gene harbouring the mutation is not predictable (7, 39, 73). However, in CMMRD associated malignancies, interestingly especially brain

tumours, MSI analysis seems to be not as trustworthy since it often shows false negative results (39). Bakry et al. (7) recently used MSI analysis on 28 CMMRD associated tumours and the corresponding normal tissue. 20 tumours turned out to be MSI stable, so this method was neither sensitive nor specific for detecting MMR defciency especially in non-gastrointestinal tumours (7). Exact reasons for the obtained deficits of this method however are not understood well enough to date (39, 75). Even if Bodo et.al. (35) suggested a new and promising combined pre – screening diagnostic method (MSI and Lymphocyte Tolerance to Methylating Agents) just recently (see (35)), identifying the both germline mutations genetically, preferably using targeted gene mutation anlysis, remains the most reliable way to confirm CMMRD. Genetic counselling and further possibility of genetic testing of patients as well as their family members, which is only initiated with informed consent, should at all times be performed based on shared decision making (36, 39). In addition, the emotional burden that comes with the suspection or further diagnosis of inherited cancer on top of facing a malignancy in a child should always be taken into consideration (40).

1.3.6 Surveillance, therapy and prognosis in CMMRD patients

As the majority of CMMRD patients die from maligancies particularly early in life, it appears obvious, that surveillance for these same maligancies and the earliest detection possible, currently consitutes the only way to achieve an improvement in prognosis (61).

Surveillance for colorectal carcinomas in LS patients has been found to be extremely advantageous and efficient in reducing mortality associated with malignancies, more specifically by more than 60% (61, 76). The same approach was followed when Vasen et al. on behalf of the European consortium „Care for CMMR-D “, proposed Guidelines for surveillance of CMMRD patients in 2014 (61). They suggested specific surveillance procedures for the most common cancers occuring in CMMRD, respectively starting with the youngest age that malignancies are currently known to occur, as shown in **Figure 2**.

Type of cancer	Lower age limit	Procedure/interval
Brain tumours	From age 2 years	MRI, 1×/6–12 months
Digestive tract cancer		
SBC	From age 10 years	VCE, upper gastrointestinal endoscopy*; 1×/year
CRC	From age 8 years	Ileocolonoscopy; 1×/year;
Haematological malignancies		
NHL/other lymphoma	From age 1 year	Clinical examination 1×/6 months Optional: abdominal ultrasound 1×/6 months
Leukaemia	From age 1 year	Blood count 1×/6smonths
LS-associated cancers†	From age 20 years	Gynaecological examination, transvaginal US, Pipelle curettage (1×/year), Urine cytology, dipstick (1×/year)
All cancers	Parents and patients should be advised to contact their doctor in case of unusual signs or symptoms. A pamphlet should be available with information about the signs/symptoms that may occur.	

*At the same time as colonoscopy under general anaesthesia.
†See: Revised guidelines for the clinical management of Lynch syndrome: HFA Vasen *et al Gut* 2013.
CMMR-D, constitutional mismatch repair-deficiency; CRC, colorectal cancer; LS, Lynch syndrome; SBC, small bowel cancer; NHL, non-Hodgkin lymphoma; VCE, video capsule endoscopy.

***Figure 2: Surveillance protocol for CMMRD patients**, taken from Vasen et al. 2014 (61)*

As a matter of fact, several publications on surveillance in CMMRD patients exist (e.g. see (7, 42, 77) and recommendations do differ slightly when it comes to starting age and intervals of performing surveillance procedures. However, publications do agree, that the exact value and efficacy of performing these procedures in the context of surveillance programms are currently unknown and need further examination. Surveillance for brain and especially colon tumours though already showed success and might be effective, albeit to an unknown extent (7, 42, 61, 77). Additionally, newer publications propose the whole body MRI (WBMRI) as a surveillance method, like it is suggested in Li Fraumeni syndrome (16), especially since our knowledge considering the tumour spectrum of CMMRD patients is constantly increasing (44). Heterozygous family members should be surveilled according to existing evidence based Lynch guidelines (14, 78).

Therapy of CMMRD is chosen according to the malignancy that is present and there are currently no guidelines for optimal treatment available. However, chemotherapy is often the therapy of choice in childhood malignancies. Here it is of significance to note, that in CMMRD patient´s efficacy of certain chemotherapeutic agents might be reduced because of resistance whereas cytotoxicity is possibly increased because of the existing defects in MMR. Like already mentioned MMR deficient cells tend to tolerate DNA damage to a greater extent. Their resistance to O^6 – Methylating agents like Temozolamide, often used in Glioblastoma treatment, was shown in various cell line and mouse model studies and this could result in therapy failure in CMMRD patients (27, 36, 61, 79, 80). Another point that causes worries is that some agents, amongst others temozolamide, cause somatic mutations that cannot be repaired in CMMRD patients because DNA damage signaling is not working properly (27). This could be proved in a patient that accumulated somatic mutations after treatment with temozolamide (61, 81). These mutations, that are left

unrepaired, however might increase the risk to develop another, then therapy induced, primary tumour as a result (36). Busulfan has also been found to cause similiar mutations in vitro (79) and clinical resistance to cisplatin and thiopurine is also being suggested (61). Scott et al. discussed chemotherapy in CMMRD patients in 2007 (51) and Vasen et al. (61) evaluated the response of those to certain chemotherapeutic agents in 2014 and could show that overall brain tumours respond the worst. It is nevertheless arguable to date wether agents, such as the ones discussed above, should be contraindicated in CMMRD patients (61). However early detection of malignancies might not only improve prognosis but leave time to chose the appropriate agent for the respective patient (51, 61).
Even though the actual cancer risk and penetration are currently unknown and might as well be overestimated as our knowledge is based on publications of a possibly biased cohort, CMMRD seems to be a highly penetrant syndrome with a prognosis much worse than we know it from LS. This is attributable to the types of malignancies that occur in CMMRD and the immense risk of developing multiple malignancies (7, 36, 61). Most patients end up dying from Glioblastoma (61) and overall survival is in general found to be extremely poor (7).

1.3.7 Other findings in CMMRD patients – a link to the immune system

Apart from malignancies and other premalignant or nonmalignant clinical features and hints CMMRD patients have interestingly been described to show mild abnormalities concerning their immune system, more specifically their methods to ensure diversity in the same (36, 82). The MMR system actually has various jobs apart from guaranteeing fidelity in DNA replication. Amongst others, it plays a role in immunoglobulin class switch recombination (CSR) and somatic hypermutation (SHM) like already mentioned (21, 34). As stated by de Miranda et al. (82), DNA repair, in which MMR constitutes one of the six main mechanisms, therefore represents a link between the immune system, immunodeficiency and cancer (20, 82).

1.4 DNA repair and the Immune system

Genetic diversity, the „basis of evolution “, plays an important role in our immune system as we have and always had to deal with a huge variety of pathogens in order to ensure survival (82, 83). Our immune response against potential pathogens distinguishes between a rapid innate and an adaptive immune response, with the

latters key features being specificity and capability of generating an immunologic memory. B and T lymphocytes play leading roles in that complex process but are dependent on DNA repair and damage response protein complexes to properly fulfill their duty in the fight against the plethora of pathogens we are exposed to daily (82, 84-86). The importance of DNA repair and maintainig genomic stability in the pathogenesis of cancer and cancer predisposition syndromes was already dicussed. Impaired DNA repair though can, caused by its participation in lymphocyte development, also lead to primary immunodeficiencies (PID), which in turn often implies a strong predisposition to develop malignancies as DNA repair, and that is the point, is not functioning properly.

This is all caused by genomic instability and a lack of diversity (20, 82).

Diversity, provided by different molecular mechanisms that occur during maturation of lymphocytes, shall represent the centerpiece of attention in this chapter as it is crucial for an adaptive immunresponse and therefore central in a properly functioning immune system (82, 87).

1.4.1 Molecular background: What we need is Diversity

1.4.1.1 T cell and B cell receptors-Immunoglobulins

T and B Lymphocytes need to recognize a huge variety of antigens. In order to do that they need antigen-recognition molecules, respectively called T cell receptors (TCR) and B cell receptors (BCR) or immunoglobulins (Ig).

Antibodies, the secreted form of B cell receptors, are able to recognize and bind a plethora of antigens from different pathogens and once bound recruit other cells and molecules in order to destroy the just bound pathogen. Immunoglobulines,Y shaped dimers of heterodimers with a connecting disulfide bond (88), are composed of polypeptide chains: two identical light chains and two identical heavy chains. Each of these chains consists of a variable and a constant region. The five main classes of immunoglobulins – IgM, IgD, IgG, IgA and IgE all activate different effector functions and are defined by the structure of their heavy chain more specifically the constant region (85). The heavy chains are accordingly designated by the greek letters μ, δ, γ, α and ε. IgG has 4 subclasses (1-4) in humans and furthermore represents the most frequently occuring immunoglobuline (85). Light chains exist in two different types that are to date not found to differ in function, namely lambda (λ) and kappa (κ). The variable region varies -as the name implies- exceedingly

between different antibodies, which makes them as diverse (85). The variable region of the light chain (VL) and the variable region of the heavy chain (VH) create the variable region of the antibody which is capable of binding antigens. The constant region of the antibody consists of the constant regions of heavy and light chain (CH, CL) and plays the leading role in interaction with effector molecules and cells (85). From the functional point of view an antibody consist of three important pieces or fragment - two so called Fab (Fragment antigen binding) fragments composed of the V regions and binding antigen and one Fc (Fragment crystallizable) fragment consisting of the C regions and performing the function of interaction (85).

T cell receptors, reminiscent of Fab fragments of antibody molecules, consist of 2 chains (α/β or γ/δ), each of which is composed of a C and a V domain, with the latter being capable of recognizing, but not binding, antigens (85, 88). TCR are associated with the cytoplasmatic protein CD3 and their diversity can be determined using the fact, that on each α/β T cell, only one β chain can be exprimed. Therefore, the variable region of the β chain is used to evaluate the, so called, T cell V-beta repertoire (89-92).

1.4.1.2 Achieving diversity of our immunoglobuline repertoire

A place where diversity is needed, is the antigen binding receptor. B and T lymphocytes have to express a multitude of different receptors on their surface to recognize a multitude of antigens as they are supposed to do (82). The generation of about 10^{18} genetically different cells with respectively different and unique receptors is required to recognize the upcoming unique antigens. But how does this variation in receptors come about (18)?

During lymphocyte development antigen binding receptor genes go through genetic combinatorial mechanisms required to provide antigen receptor diversity needed for an adaptive immune response, in which we know variety is crucial (87, 93).

First, V(D)J recombination takes place in the variable region of BCR and TCR, a somatic event that is theoretically capable of producing 10^{11} different antigen receptors. This process is followed by class switch recombination (CSR), mainly in the constant region of B- cells, resulting in the production of different isotypes of antibodies and finally point mutations in the variable region of immunoglobulines are introduced to improve antigen affinity of the same, which is referred to as somatic hypermutation (SHM) (82, 94). These genetic rearrangements are initiated by

creation of DNA lesions, which in turn activates DNA repair mechanisms to repair the just created lesions. Mutations affecting DNA repair pathways can lead to the interruption of these diversity- achieving processes that are needed for a functioning immune response (94).

1.4.1.2.1 V(D)J recombination

T cell receptors and B cell receptors or immunoglobulins are composed of a constant and a variable region, with the latter being assembled of a V, D and J segment (95). V(D)J recombination occurs at an early stage of lymphocyte maturation and refers to somatic DNA rearrangement of the variable (V), diversity (D, only in heavy chains) and joining (J) segments of B cell receptors and T cell receptors (82). B cell receptor recombination occurs in the bone marrow whereas T cell recombination occurs in the thymus. Between the steps of V(D)J recombination, which will be discussed in the following, precursors of lymphocytes experience massive proliferation that comes along with replication intermediates which in turn is resolved by the so called Bloom Syndrom protein (18).

Recombination signal sequences (RSS), noncoding DNA sequences, are always located adjacent to the coding sequence of V, D and J segments and are recognized by the enzyme complex that carries out VDJ recombination, the VDJ recombinase, in order for recombination to take place at the correct location (85, 93). RSS are composed of conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) sequences separated by a nonconserved region, known as the spacer, that consists of either 12 or 23 basepairs and therefore distinguishes the two existing classes. According to the so called „12/23 rule“, efficient joining of gene segments typically involves a 12 basepair RSS and a 23 basepair RSS (85). Initially, the lymphocyte specific factors RAG1, RAG2 (recombination activating genes) are binding to an RSS flanking the sequences to help them join. The binding of two RAG complexes to two RSS forms a synaptic complex, which is important to facilitate the cleaving step that is following. RAG1 and RAG2 cleave DNA between the RSS and the coding sequence, leading to double strand breaks (DSB) (85, 93). This activates DNA damage response proteins like ATM, NBN or 53BP1. Members of the Nonhomologous end - joining (NHEJ) pathway, recruited by the DNA damage response proteins, are under obligation to deal with the arising DSB. The NHEJ pathway consists of numerous proteins, seven of which are considered essential: XRCC4, Ku70 and Ku80, DNA ligase4, Artemis, DNA –PKcs and Cernunnos.

Deficiencies in any of the just mentioned proteins may lead to severe immunodeficiency, which highlights their importance (82). The cuts mentioned occur in two steps, with the endonuclease activity of the RAG complex first creating nicks in a DNA strand neighboring the heptamer and then cleaving the second strand generating a hairpin on the coding end (85, 95). The resolving of the cut ends, however, takes place in marginally different ways (85, 95). Ku, a heterodimer of Ku70:Ku80, essential proteins, binds the signal ends but does not modify them (85, 95). Subsequently, the ligation of the signal ends, carried out by the DNA ligase, which forms a complex with a repair protein called XRCC4, precisely forms a, so called, signal joint (85, 95). Ku also binds the coding end followed by a DNA-PK (DNA dependent protein kinase): Artemis complex joining and opening the hairpin by using the nuclease activity of Artemis, yielding a flush or single strand DNA end (85, 95). Terminal deoxynucleotidyl transferase (TdT) and exonuclease activity then process those DNA ends by deleting and adding nucelotides (85, 95). This results in imprecise ends, which are then also ligated by DNA ligase associated with XRCC4 forming coding joints (D-J or V-DJ for heavy chains; V-J for light chains) (85, 95). This complex process shows that for generation of diversity, that is needed for an immune system reacting to a plethora of different antigens, a multitude of well-coordinated steps and proteins are needed, including the DNA repair pathways (82, 95).

1.4.1.2.2 Class switch recombination (CSR) and somatic hypermutation (SHM)

Whereas VDJ recombination occurs at a very early developmental stage in B and T cells, class switch recombination and somatic hypermutation are processes that only occur in antigen receptor genes of mature B cells after CD40 activation (82, 94, 96).

Those maturational modifications in immunoglobulin genes, epitomizing a response to antigen stimulation or T cell activation, take place in the germinal centres of secondary lymphoid organs before B lymphocytes meet their matching antigen and ensure optimization of the immune response (18, 94, 97). B cells, again, proliferate extensively and accumulating replication intermediates are anon resolved by Bloom syndrome protein (18).

Class switch recombination occurs in DNA segments referred to as switch regions and is required to produce the different antibody isotypes, that are determined by

the constant region of their heavy chain (CH) (97). However V(D) J specifity and thus antigen affinity is not changed by this important maturation process (34).

At first a μ heavy chain (Cμ) is expressed. By antibody class switching it can though be replaced by a γ,δ or ε heavy chain (Cγ, Cδ, Cε), all of which are constant gene segments located downstream of Cμ, by excising the sequences inbetween. CSR is again, like VDJ recombination, initiated by induction of a DNA lesion. Activation-induced- cytidine deaminase (AID), which is well known to play a leading role in antibody maturation, creates mismatches by converting cytosines in switch regions (S regions), located 5´of each CH exon, and variable regions into uracils via deamination of both the donor and the acceptor S region (82, 94, 97). Those U/G mismatches are subsequently recognized by members of the base excision repair (BER) pathway such as Uracil N- Glycosylase (UNG) and apurinic/apyramidinic endonuclease (APE1) and members of the MMR pathway (MLH1/PMS2, MSH2/MSH6), that were already discussed in this thesis (34, 82, 98).

UNG removes the uracils that were just added by AID and thus leaves an abasic site which is subsequently repaired by the endonuclease APE by incising the phosphate backbone of DNA which ultimately results in distributed single- strand DNA breaks (34, 97, 99). If the single-strand breaks on the opposite strand are not too far away this leads to a double strand break without further ado. If they are, however, the MMR system is required to convert single-strand breaks into double-strand breaks in switch regions (97, 100). Those double strand breaks are then, as we know it from V(D)J recombination, repaired by members of the NHEJ pathway to open the way for the intended replacement of Cμ, which is located next to the Ig variable region. The MMR proteins can, in alternative to the BER pathway, also recognize U/G mismatches and generate nicks surrounding the mismatch (94). However, it is suggested that the MMR system plays more of a backup role in the process of recognition and introduction of nicks whereas converting single strand breaks into double strand breaks seems to constitute their main role (34, 94, 97). It could be demonstrated in UNG-MSH2 double knockout mice that MSH2 and MSH6 recognize DNA mismatches indtroduced by AID, when UNG is absent (34, 101). In addition, underlining the suggested main function of MMR in CRS, Schrader et al. (100) could show, that in switch regions of MMR deficient B cells fewer double-strand breaks were detectable than in the compared wild type B cells (97, 100).

Furthermore, less blunt DSB were detected in PMS2 deficient B cells than in MSH2 deficient B cells of mice (34).

Somatic hypermutation, another B cell specific process, increases the antigen affinity, but again, as we know it from CSR, the antigen specifity stays the same (34, 82). SHM is, like CSR, initiated by AID, which therefore plays a main role in both processes (88, 97). Muramatsu et al. (102) described the phenotype of AID-/- mice in 2000 and could thus demonstrate that mice require AID not only for CSR but also for SHM (102). Revy et al.(103) could furthermore show that AID deficiency results in Hyper-IgM (HIGM2) syndrome, a primary immunodeficiency, caused by non-functioning CSR and SHM (103). In addition to the initiation of both the processes CSR and SHM, which constituted a groundbreaking discovery, Arakawa et.al could show in 2002 (104), to even go one better, that disrupting the *AID* gene in the chicken B cell line DT40 results in blockage of Ig gene conversion, a process resembling SHM, including the introduction of nucleotide changes in variable regions. *AID* is therefore important in all the B cell specific modification occuring in Ig genes (104, 105).

AID, the master gene, initiates SHM by introducing point mutations into variable regions of Immunoglobuline genes. It deaminates cytidine residues and again creates U/G mismatches, that are processed by DNA repair mechanisms (DNA polymerase, BER, MMR) (88). The repair of SHM induced DNA breaks is fallible and depends greatly upon MMR and error prone DNA polymerases (34). In comparison to CSR, processing of mismatches in SHM ultimately generates point mutations (82). The role of the MSH2-MSH6 complex in SHM is the recognition of U/G mismatches and the subsequent recruition of EXO1 and polymeraseη. Exact functions of *MLH1/PMS2* in SHM however remain subject of further studies (34, 106).

1.4.1.2.3 B cell maturation and surface markers overview

After discussion of the rearranging mechanisms (VDJ recombination, CSR and SHM) that occur during maturation of B cells in Ig loci, this chapter shall give a short overview of the development itself. B cell development has been the centerpiece of

a huge amount of studies (see (107)) and this chapter does not by any means imply any aim to clompleteness of this complex process.

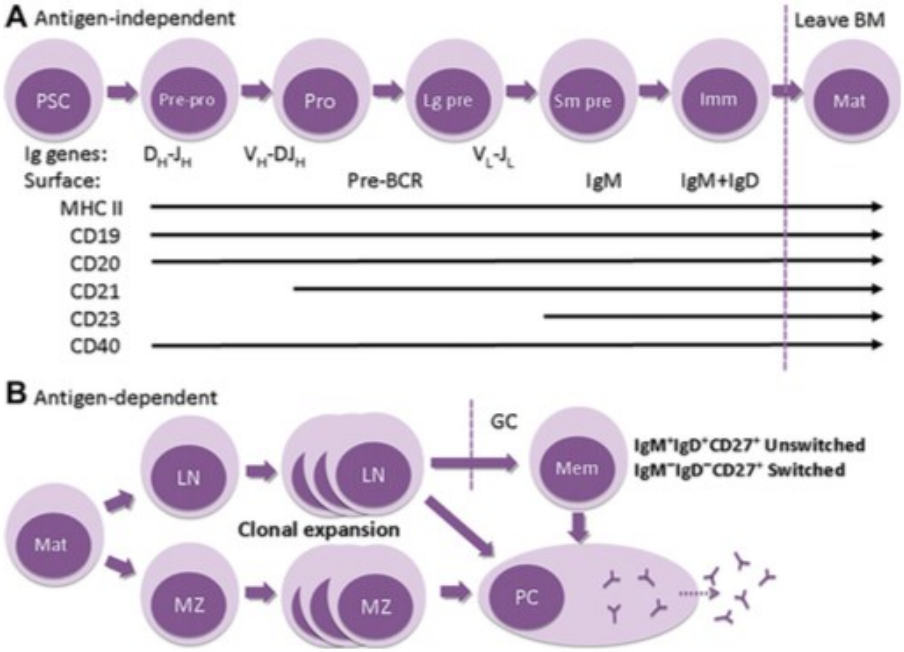

Figure 3: B cell development, *taken from Bonilla and Oettgen 2010(84), PSC= pluripotent stem cell, lg=large, sm=small,Imm=immature, Mat=mature Mem=memory*

B cells emerge from hematopoetic stem cells, located in the bone marrow (BM). The antigen-independent phase, implying that no contact with antigen is yet given, consists of several stages of development as shown in **Figure 3**. The aim is to achieve antigen specifity via V(D)J recombination of heavy and light chains. As soon as the immature stage is reached, B cells leave the bone marrow and when IgD in addition to the already expressed IgM appears on the surface they are referred to as mature or naive B cells. The peripheral antigen dependent development depends on antigen, that can be encountered by mature B cells in lymph nodes (LN). Activation can make them short-lived plasma cells (PC), that produce antibodies. Memory cells, however, are mostly build in germinal centers (GC). Activated memory cells can turn into long lived plasma cells. The presence of the surface marker CD27 distinguishes activated from non-activated cells. Cells that have not undergone CSR remain IgM and IgD on their surface and are thus called unswitched while so called switched cells do not longer express IgM and IgD. There is another pathway including cells in the marginal zone (MZ) of the spleen which however does not generate a lot of B cell memory (84). **Figure 3** also shows surface markers expression during developmental stages such as CD19 which is expressed by all

cells of the B lineage and belongs to the immunoglobuline superfamily (107). The term CD, meaning „clusters of differentiation “, was given in 1982 to facilitate the classification of monoclonal antibodies then and is now used to classify all cell surface molecules that can be used for immunophenotypic identification of cells (107, 108). The first described B cell specific molecule was originally termed B1 and is now known as CD20 (107).

Surface phenotype	B-cell subset
$IgM^{+}IgD^{-}CD27^{-}$	Immature
$IgM^{+}IgD^{+}CD27^{-}$	Naive
$IgM^{+}IgD^{+}CD27^{+}$	Marginal zone (unswitched memory)
$IgM^{-}IgD^{-}CD27^{+}$	Germinal center (switched memory)*
$CD38^{low}CD21^{low}$	Uncharacterized†
$CD38^{high}IgM^{high}$	Transitional (activated)
$CD38^{high}IgM^{-}$	Plasmablast

*Reduction in this population is associated with several complications of common variable immunodeficiency.
†Expansion of this (thus far) otherwise uncharacterized population is seen in patients with autoimmune diseases, such as lupus, and in patients with common variable immunodeficiency with autoimmune complications.

***Figure 4: B cell subpopulations in peripheral blood**, taken from Bonilla and Oettgen 2010 (84)*

Figure 4 shows that expression of surface markers differs in the subpopulations of B cells found in peripheral blood (84).

In general, membrane molecules CD19, CD20 and CD21 are expressed on B-lymphocytes in peripheral blood and they are referred to as markers for B-cells (109, 110).

CD27 helps us, as already mentioned above, distinguish activated B cells from other B cells, as this surface marker is only expressed after antigen contact. B memory cells can be detected by this same marker, albeit the antigen dependent process of mature B cells turning into B-memory cells is to date subject of research (109, 111, 112). CD38 is another important marker when it comes to B cell subpopulations. It is a marker, that is measurable on precursor B cells and naive B cells, but, in comparison to CD27, not on B-memory cells. It is, however caused by the further CD38 expression that occurs during the germinal center reaction, that this surface marker is again easily measureable on plasmablasts, that indeed show a high CD38 expression (109, 110). Surface markers/antigens can be detected with Fluorescence- activated cell sorting (FACS) and make a distinction of B cell subpopulations possible (109-112).

1.4.2 Immunodeficiencies caused by DNA repair defects

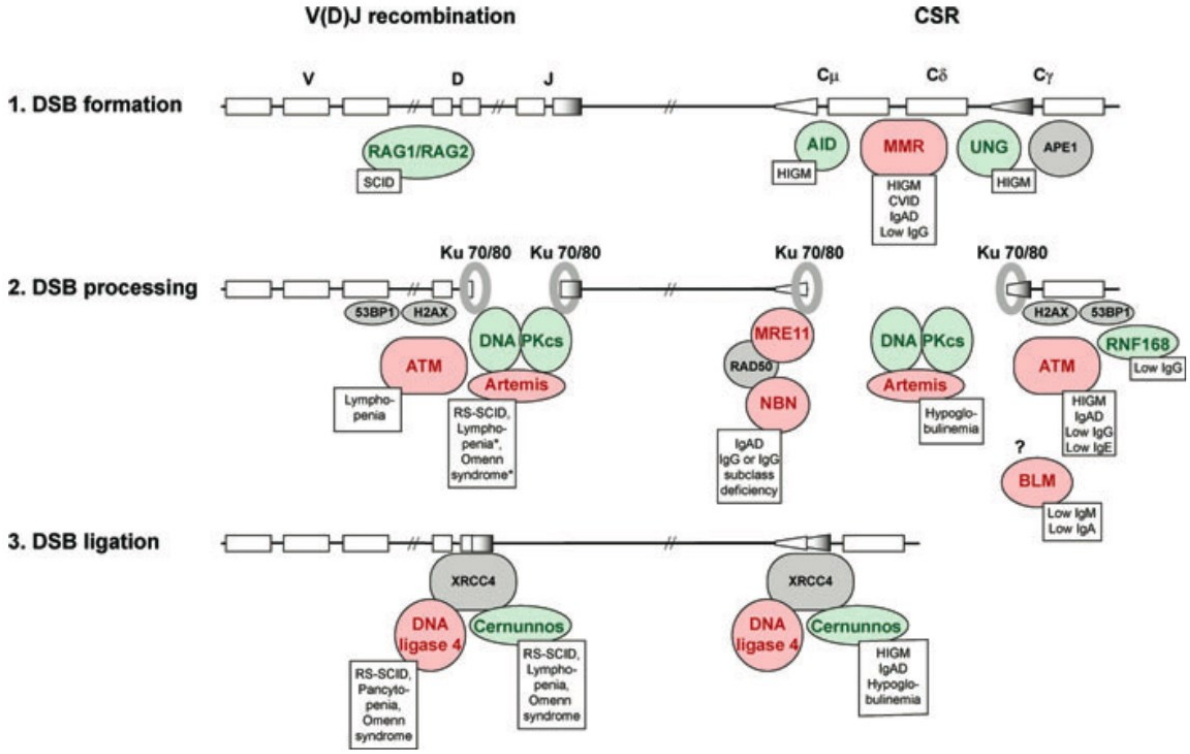

***Figure 5:Immunodeficiencies caused by DNA repair defects**, taken from de Miranda et al. 2011 (82)* Proteins in green have not yet found to be associated with cancer whereas proteins in red are associated with a predisposition to cancer*

The complex mechanisms lymphocytes undergo during maturation depend greatly, as already stated above, on DNA repair, including MMR- the pathway representing the centerpiece of our interest since reparation of targeted DNA damage that is introduced during those mechanisms is required (18, 82). Consequently, the fact, that defects in genes concerning DNA repair also affect the immune system, does not appear surprising. **Figure 5**, as shown by de Miranda et al. (82) in 2011, gives an overview of the so called primary immunodeficiencies (PID), defined as quantitative or qualiative defects concerning cells or other elements of the immune system, deriving from defective DNA repair (82, 113).

PID's that occur in about 1 out of 10000 births, with widely ranging prevalence estimates (1:1200 – 1:400000), genetically predispose to infections with an increased severity and frequency, autoimmunity and malignancies. The latter predisposition, which especially affects patients with defective cellular immunity, is explainable by the decreased alertness of the immune system towards cancer cells

(82, 114, 115). If the respective cause of PID is an underlying DNA repair deficiency, this logically adds up to the risk of developing cancer (82).

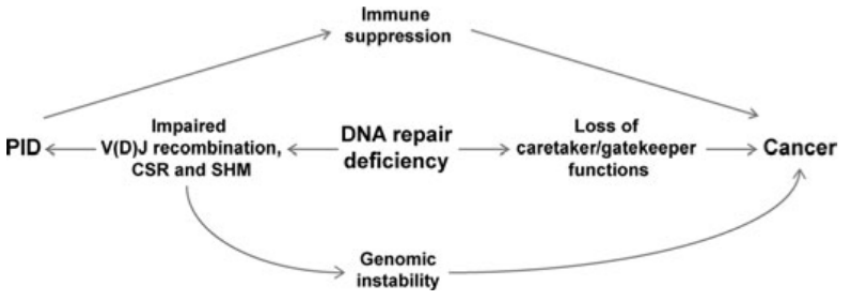

***Figure 6: Relation between DNA repair deficiencies, PID and cancer**, taken from De Miranda et al. 2011(82)*

Figure 6 shows the relation between DNA repair deficiencies, PID and cancer. Genomic instability, that can be caused by defective mechanisms in antibody diversification, in addititon to the impaired immune system in general, contribute to the predisposition towards cancer in patients with DNA repair deficiencies deriving from impaired V(D)J recombination, CSR and SHM, that suffer from PID (82, 114, 116-118).

It is apparent from **Figure 5**, that deficiencies of different DNA repair genes result in respectively different PIDs. It depends on which role the affected genes play in the maturation process of lymphocytes. There might as well be more than one process affected, as it is well known, that VDJ recombination, CSR and SHM have some DNA repair pathways, they are dependend on, in common (82).

V(D)J recombination is crucial for development of T as well as B lymphocytes. Therefore, defects of RAG1 or RAG2, that initiate this process but do stricty not belong to the DNA repair pathway, result, as one would assume, in the most severe immunodeficiency, referred to as severe combined immunodeficiency (SCID). Patients suffering from this condition are characterized by absence of both B and T lymphocytes, which if not treated by hematopotic stem cell transplantation, often leads to death within their first year of life (18, 82, 119).

Defects can also occur in proteins associated with sensing and processing the introduced DSB. ATM mutations lead to Ataxia teleangiectasia, an autosomal recessive disorder, presenting with impaired V(D)J recombination and CSR. Additionally, a predisposition to lymphomas, leukemia and other solid tumours is present in this patient population (18, 82, 120). Artemis, as part of the NHEJ pathway, is among other things crucial for V(D)J recombination. This explains why null mutations in the Artemis encoding gene DCLRE1C result in a condition referred

to as radiosensitive severe combinded immunodeficiency (RS-SCID). The clinical phenotype can, apart from the additional radiosensitivity, not be distinguished from the one found in RAG deficient patients (18, 82, 121). RS-SCID is also found in a few patients with LIG4, which encodes for DNA ligase 4, mutations and all patients with DNA-PKcs deficiency decribed so far developed RS-SCID as well (82). Hypomorphic mutations in DCLRE1C lead to Omenn syndrome, which is also found in patients with hypomorphic RAG mutations and referred to as „leaky SCID “, hypoglobulinemia and lymphopenia (18, 82, 122). Artemis deficiency and DNA ligase 4 deficiency are moreover associated with cancers, with EBV positive lymphomas being the most notably (82). Deficiency of Cernunnos, another NHEJ pathways protein, has only been described in eight patients so far. It is not known to be associated with cancers, but with radiosensitivity and different phenotypes of immunodeficiency (18, 82).

Diffferent conditions presenting with impaired DSB initiation, recognition, processing and ligation as described above all show defects concerning early B cell development, i.e. V(D)J recombination. On the other hand, late B cell development, namely CSR and SHM can also be impaired, resulting in rare PIDs referred to as immunoglobulin class- switch recombination deficiencies (Ig-CSR-Ds) (96, 113).

1.4.2.1 Immunoglobulin class- switch recombination deficiencies (Ig-CSR-Ds)

Defective CSR goes hand in hand with defective switched isotype (IgG/IgA/IgE) production. Different underlying molecular defects can be the cause for this rare condition and their role in B cell maturation predetermines wether SHM is affected as well (96).

A disorder which presents with all the hallmarks of impaired CSR is Hyper –IgM syndrome (82). Patients suffering from HIGM show normal or high levels of IgM, whereas levels of IgG and IgA are decreased or absent. This in turn leads to a predisposition towards bacterial infections (82, 96). The estimated prevalence of this condition is 1/500000 in Caucasians (113). HIGM becomes apparent in early childhood when patients present with severe and recurrent infections such as respiratory tract infections, gastrointestinal infections and sometimes even infections caused by opportunistic pathogens like Pneumocystis jiroveci or Cryptosporidium. However, HIGM still represents a condition that is very likely to be

overseen by clinicians for a long time (18, 113). Even though CSR deficient patients don´t produce IgG antibodies against pathogens or vaccines, isohemaglutinin levels and anti-polysaccharide IgM antibodies are found to be normal. As one would assume, switched IgG+ or IgA+ B lymphocytes are not detectable in peripheral blood in patients suffering from defective CSR. CD27+ B cell levels are either normal or extremely low, depending on the underlying defect. CSR deficiencies show a variety of underlying molecular defects, from defects in Tcell- Bcell cooperation, such as defects in CD40, to intrinsic B cell defects, such as AID deficiency to DNA repair defects (96). As shown in **Figure 5**, HIGM can be caused by AID deficiency (103), resulting in an autosomal recessive disorder characterized by impaired CSR as well as SHM (123), or other proteins that are crucial for CSR such as UNG, ATM, NBN or MMR proteins (34, 51, 82, 124). This enormous variety of genetic causes is why nomenclature distinguishes different types of HIGM (113).

Some types of HIGM appear similiar to Combined variable immunodeficiency (CVID), a heterogenous disorder presenting with absent or decreased levels of IgA, low levels of IgG (<3g/l) and normal or, as described in 50% of patients, decreased levels of IgM. In comparison to the exceedingly low prevalence of HIGM, CVID is estimated to occur in 1/20000-1/50000 of the Caucasian population. Patients suffering from CVID are prone to recurrent bacterial infections such as respiratory and GI infections which is attributable to hypogammaglobulinemia (113, 125). There are several underlying molecular defects that have been discovered so far (e.g. ICOS, TACI, CD19) and it is suggested by Pan-Hammarstrom et al. (113) , that there will be a nomenclature distinguishing different types of CVID in the near future (113). Out of the DNA repair defects that are known to cause immunodeficiencies, CVID is only described in MMR deficient patients as apparent from **Figure 5** (82, 126).

The immunodeficiency known as the most common one in the western world is IgA deficiency, with a serum level of 0,05g/l defined as the maximum for diagnosis, normal serum IgM and normal or high serum IgG, especially IgG1 and IgG3. This relatively common PID is described to occur in 1 out of 600 individuals. It must be noted, however, that 2/3 of the individuals affected show no increase in infection frequency whatsoever. The remaing 1/3 suffers from recurrent infections of the upper and lower respiratory tract. The genetic cause of this relatively common PID remains somewhat elusive. But, among others, several DNA repair proteins, namely

NBN, LIG1 and finally, as the centerpiece of our attention, MMR proteins were described to contribute to this defect (113, 125).

There are several autoimmune diseases, such as systemic lupus erythematosus, Sjögren syndrome, rheumatoid arthritis, celiac disease, autoimmune hemolytic anemia and others, that were found to be associated with CVID and IgAD. Goshen et.al. (127) employed a large panel of autoantigens in their study conducted in 1989 and could show that 39% of the sera of healthy subjects with detected IgAD harbor polyreactive antibody activity, with the majority of the autoantibodies being from the IgG isotype (127). A predisposition to autoimmune diseases and symptoms is therefore suggested ever since in patients with antibody deficiencies (125).

Table 4: Laboratory findings in CVID and IgAD

Parameter	CVID	IgAD
Serum IgM levels	Low/normal	Normal
Serum IgG levels	Low	Normal/high (esp. IgG1, IgG3)
Serum IgA levels	Low	Low
Secretory IgA	Low	Low
Surface IgM+ cells	Normal	Normal
Surface IgG+ cells	Normal	Normal
Surface IgA+ cells	Normal	Low/Normal
Autoantibodies (anti- IgA)	20-30%	30-40%
Lymphocyte CD markers	Normal	Normal
Plasma cells (bone marrow)	Low	Normal

modified from Hammarstrom and Smith 2007(125)

Table 4 shows a summary of laboratory findings in patients with CVID and IgAD.

1.5 Primary immunodeficiencies (PID) - Warning Signs

In the 1960s Bruton first described a condition referred to as X-linked agammaglobulinemia. Since then, more than 130 PID, of which some have already been discussed above, have been described. Although the underlying causes differ from condition to condition, the result is an individual that is prone to more severe and more frequent infections, which are often not well curable and can end fatal. To recapitulate, this chapter should give a short overview of signs and symtoms that PID have in common and that may help to lead into the direction of diagnosis.

As there is a massive amount of different PID, that are often not diagnosed early and proper enough, so called “PID warning signs” have been summarized and

published, in order to raise awareness and achieve prompt recognition and prevention from death caused by infections (115, 128, 129).

***Figure 7: The 10 Major Warning signs of PID**, taken from Waltenburg et al. 2010 (115)*

Figure 7 displays the 10 warning signs of PID, that can be summarized as increased frequency, increased severity and harder cure of infections as well as reduced thriving and family history of PID. If two or more of these signs apply to an individual, the presence of a PID should be taken into consideration by the physician (115). Furthermore, delayed healing of the omphalos and reactions or complications after live vaccines represent worrying signs in an infant. Live vaccines should thus not be administered and are contraindicated in patients with severe immune impairment. Even if, and that is another observation featured in PID, the response to vaccinations is generally reduced in PID patients, administration of killed vaccines is still recommended (130, 131).

Signs of immunodysregulation are granulomas, autoimmunity, recurrent fever, lymphoproliferation, ekzema as well as inflammatory bowel disease. Reduced thriving in an infant is, as displayed above, one of the major warning signs of PID, and often goes hand in hand with diarrhea and weightloss, another symptom that is often observed in affected imdividuals. Amongst others, these clinical signs can all be referred to as warning signs for PID. They should help to improve prompt and proper diagnosis of this various group of diseases (130-133).

1.6 Immunological findings in CMMRD patients

In 2002, 3 years after the first two reports on CMMRD patients were published (45, 46), Whiteside et al. (134) described a 24 month old child, that was noted to have multiple CALMs, presenting with failure to thrive and a gastrointestinal infection. This patient could subsequently be diagnosed with ALL and IgA deficiency and an underlying homozygous germline mutation in *MSH2* was furthermore detected (134). Since this first immunological finding in a CMMRD patient, 13 CMMRD patients were found to show decreased IgA and/or IgG2/4 levels or, particularly in young children, high IgM levels, resembling HIGM (34, 39, 51, 123, 124, 134).

1.6.1 Humoral immunological phenotype of CMMRD patients

The role MMR plays in CSR, as a backup for AID induced DNA mismatch recognition and a converter of SSB, as well as in SHM, as a repairer of DNA breaks, was already discussed in this thesis and could initially be described based on knockout mouse models used in several studies conducted (34, 123, 135-137).
CSR and SHM were actually shown to be less efficient in MSH2, MSH6, MLH1 and PMS2 deficient knockout mice, which again highlights the suggestive contribution MMR proteins make to antibody maturation and explains why these same Mut homolgues make suitable candidates for Ig immunodeficiency occuring in humans as well (138, 139).
IgA deficiency, as the most common PID with a selictivity that is not quite understood yet, was described in 1 CMMRD patient carrying a biallelic *MSH2* mutation (134) and in 3 CMMRD patients carrying a biallelic *MSH6* mutation (51, 124), one of which also showed IgG2 deficiency (51), to date. Furthermore, defects in MLH1 and MSH2, which are known to be MMR proteins that are found mutated in HNPCC and CMMRD patients, as well as genetic variants of MSH5, representing another MutS homologue, were found to contribute to IgAD and CVID in humans (82, 126, 138).
PMS2, the fourth and foremost MMR protein associated with CMMRD, has also been associated with impaired CSR. In particular, in vivo and in vitro isotype switching and switch recombination defects, indicated by increased microhomology across switch junctions, were detected in PMS2 deficient mice. However, SHM was not affected in PMS2 deficient mice, as the unaffected Hypermutation profile demonstrated (34, 140, 141). Peron et al. (34) could achieve similiar results in a study conducted in 2008. A homozygous *PMS2* mutation was detected in three

patients with IgCSR deficiency and Peron et al. (34) could show that CSR was partially defective in vivo and clearly impaired in vitro, with all other known causes of CSR defects ruled out. SHM generation was once more, concurrent with the results in mice (140), not affected, as indicated by a normal nucleotide substitution pattern (34, 142). However, peripheral blood CD27+ memory B cells were found to be low in 2 patients. Serum IgM was normal or elevated, IgG2 and IgG4 was low in all patients and reduced total IgG and/or IgA was found in symptomatic patients. IgG and IgA were found to rise with age, which might be explained by the accumulation of long lived plasma cells (34, 142).

Despite the fact, that CSR and SHM seem to have a lot in common, PMS2 deficiency was to date, according to what Peron et al. (34) revealed in humans, not found to hugely affect SHM. This finding is in contrast to what is known about MSH2 deficiency, which affects both CSR and SHM, from mice (34, 140). The role of MSH2/MSH6 in both the processes was confirmed in studies conducted using double knockout mice (UNG/MSH2, UNG/MSH6) (34, 143). Peron et al. (34) could furthermore reveal that MMR plays a role in DSB induction during CSR in humans by showing that the IgCSR defect in PMS2 deficient patients occured upstream of the induced DSB in S regions which is consistent with the endonuclease activity PMS2 was confirmed to have (34, 100, 123). Peron et al. (34) were therofore the first to reveal PMS2 deficiency as a cause of impaired CSR in humans, however the fact that UNG deficiency results in profoundly severe CSR defect implies that PMS2 initiated MMR does not live up to the suggestion of it being an alternative pathway to BER (34, 144).

In 2012 Gardes et al. (123) studied antibody maturation, in vivo as well as in vitro, in eight patients with biallelic *MSH6* mutations. The results revealed low serum IgG2 and IgG4 levels, low levels of switched B cells in peripheral blood, inability to undergo CRS in vitro and increased microhomology at S junctions in patient´s B cells, all of which is consistent with impaired CSR caused by the underlying biallelic MSH6 mutation. In addition, children showed high levels of IgM as an indirect sign of defective MMR whereas older patients showed normal levels of IgM, again, explainable by the compensating production of long lived plasma cells by IgG and IgA (123, 145). CSR in MSH6 deficient patients was, as Peron et al. (34) could show in PMS2 deficient patients in 2008, partially impaired in vivo and hugely impaired in vitro and MSH6 is suggested to play a role in both DSB induction and repair. In

comparison, however, to what Peron et al. (34) showed in PMS2 deficient patients concerning SHM, Gardes et al. (123) could demonstrate skewed SHM patterns, indicating SHM defects, in MSH6 deficient patients suggesting that MSH6 contributes to human SHM (34, 123).

1.6.2 Clinical immune phenotype of CMMRD patients

In general, immunodeficiencies in CMMRD patients are not found to be very distinct. The existence of both BER and MMR to deal with AID induced mismatches might be an explanation for this phenomen (82, 97). However, some of the IgA deficient patients carrying either *MSH2* or *MSH6* mutations became clinically suspicious because of recurrent infections and one IgA deficient patient also received Ig transfusions (51, 134).

None of the MSH6 deficient patients was noted to be especially prone to bacterial infections, which might be caused by other MMR proteins compensating in absence of MSH6. However, one MSH6 deficient patient was diagnosed with SLE (123).

In comparison to these findings, Peron et al. (34) could show in 2008 that sometimes a CSR deficiency represents the main symptom of an underlying biallelic MMR mutation over multiple years. Durandy et al. (142) describes, that recurrent bacterial infections primarlily led to the diagnosis of a PID. PMS2 deficiency is therefore found to result in a more distinct immunodeficiency as an observation recently revealed, reporting that 3 out of 9 PMS 2 deficient patients suffered from a clinical Ig deficiency with the need of Ig replacement therapy (34, 123, 142).

CSR deficiencies, to recapitulate, have so far been detected, at the very least in vitro, in all studied CMMRD patients (142). The lack of clinical descriptions so far and clincal signs in some patients might have hindered clincicians to test Ig levels in non- suspicious patients to date (39). However, CSR deficiencies might be the most conspicious feature of CMMRD for years and patients suffering from CSR deficiencies and cancer could be even more susceptible to bacterial infections during chemo. CMMRD should therefore be considered in patients presenting with a subtle CSR deficiency with an unknown underlying cause (123, 142).

Vice versa, CMMRD patients should be considered to also suffer from an Ig deficiency regarding an optimal treatment especially during chemotherapy (39, 123, 142).To conclude, studies concerning immunological aspects of CMMRD, especially clinical features and descriptions of the clinical immune phenotype, are to date missing.

2. Hypothesis and aims of the study

The MMR system, with its main function being the detection and elimination of Non-Watson-crick base pairs and strand misalignements that arise during DNA replication, maintains genomic stability. Furthermore, it is, amongst others, needed for B cell maturation and diversity of our immunoglobuline repertoire. MMR proteins play a role in biological mechanisms, namely class switch recombination (CSR) and somatic hypermutation (SHM), that are needed to achieve this aforeaid diversity (21, 24-26). It is therefore expected and has already been shown that CMMRD, a cancer predisposition syndrome, which is characterized by defective mismatch repair caused by an underlying biallelic mutations in one of the four MMR genes *MLH1, MSH2, MSH6 or PMS2*, may lead to defective CSR and SHM. This in turn might result in conditions such as HIGM, CVID, IgA deficiency or low IgG (34, 82). Impaired CSR and SHM in CMMRD patient could thus possibly lead to clinically relevant immunodeficiencies.

Little is known about the clinical immune phenotype of CMMRD patients. Additionally, it is not fully understood to date, to which extent a defect in MMR affects lymphocyte maturation, differentiation, memory generation, and immunoglobulin class switch and diversity. This project addresses both the clinical and humoral immunological phenotype as well as a deep assessment of immune maturation steps of patients with CMMRD across Europe and the Middle East to increase our understanding of the role of MMR system in immunoglobulin maturation.

As the identification of new CMMRD cases makes a valuable contribution to the overall knowledge of this rare condition, a thourough clinical description of this case series is another main approach of this project.

2.1 Hypothesis:

The hypothesis of this study is, that CMMRD is associated with a detectable degree of primary immunodeficiency, in laboratory parameters as well as in correlating clinical signs, due to impaired B-lymphocyte maturation and immunoglobulin generation (resembling Hyper-IgM-syndrome/ IgAD/ IgGD/CVID)

2.2 Aims of the study:

1) Detect existence or absence of clinical signs and symptoms of immunodeficiency

2) Collect laboratory parameters of the cellular and humoral immune system

3) Analyze evidence of B cell maturation defects from laboratory data provided.

4) Analyze T cell maturation from laboratory data provided to get an impression of the whole immunophenotype of CMMRD patients

Apart from this immunological approach, clinical findings of included patients shall be evaluated and thouroghly described, since only 198 patients were reported to date, compared to previous findings to increase our knowledge of the natural history of this rare syndrome.

3. Patients and Methods

3.1 Patient collective, inclusion & exclusion criteria

A total of 11 patients' data could be included in this retrospective study. Data were collected with the inclusion and exclusion criteria reading as follows:

Inclusion criteria:

- Patients diagnosed with CMMRD both male and female, most often diagnosed upon a newly diagnosed CMMRD-linked malignoma.
- Children and adolescents were included in the study because the onset and therefore the different life threatening forms of cancer mostly occur in that period of life.
- Women of childbearing age were included because the syndrome occurs equally in men and women.

Exclusion criteria:

- genetic exclusion of CMMRD (being performed outside of the study as part of the routine diagnostic program at the Institute of Human Genetics, Medical University Innsbruck)
- missing laboratory parameters of the cellular and humoral immune system

3.2 Practical application

The study was conducted as part of the European consortium "Care for CMMRD (C4CMMRD)" (36, 78) and information about it was distributed throughout the existing network. A retrospective and descriptive evaluation of a patient series including 11 patients was conducted. Physicians from various pediatric oncology centres in Europe and the Near East who declared interest in participating in the study were provided with a checklist and questionnaire on the infectiological and clinical immunological history of the patient. (see Appendix)

This form, containing all relevant information needed for the study (i.e. questions on history, previous and current health and diseases, immunological laboratory parameters) was subsequently filled out by the physicians in charge and returned to us. There was no direct patient contact involved in the study. Anonymized patient

data was collected, entered to an electronic, password-protected database, established using Microsoft Excel © Version 2016, analyzed and interpreted regarding detectable deviations of clinical or laboratory parameters of the immune system from the physiological function.

3.2.1 Data collection/ Collected variables

Participating centers

- France: Paris, Villejuif
- Germany: Kassel, Duisburg
- Slovakia: Bratislava
- Spain: Caceres/Madrid, Toledo/Madrid
- Netherlands: Leiden
- Israel: Rambam/Haifa

provided us with information concerning both the clinical as well as the cellular/humoral immunological phenotype of CMMRD patients. Where available up to 23 clinical and 52 laboratory parameters of the cellular and the humoral immune system were collected.

3.2.1.1 Demographic & General parameters

According to the respective center the patients belong to and were treated and diagnosed at they were subsequently anonymized and internally referred to as P1 for Paris, V1, V3 for Villejuif, K1 for Kassel, D1 for Duisburg, B1 for Bratislava, M1, M2 for Caceres/Madrid, T1 for Toledo, L1 for Leiden and R1 for Rambam/Haifa for correspondence reasons. After completion of analyses and data collection, to further disguise the patients' geographical origin, patients are referred to as P1-P11 in the following and any other published material.

Furthermore, general parameters such as age at point of inclusion into the study, gender, first symptom/indication for testing and age at occurence of first symptom were noted.

3.2.1.2 Clinical information

To get an idea of the clinical immune phenotype a clinical questionnaire, as already mentioned above and attached in the appendix, was sent to clinicians in participating centers. The questions included in this form covered the patients history (i.e. family history, malignancies, premalignancies, nonmalignant features,

treatment), immunodeficiency warning signs such as family history of immunodeficiency, hospitalizations for infections, complications after life vaccines, pathological healing of the navel/ omphalos or delayed growth, past infections (i.e. frequency, severity, localization, pathogen, response to antibiotics, requirement of corticosteroids/immunosuppression), signs of autoimmunity or immunological dysregulation such as granuloma, autoinflammation /relapsing fever lymphopathy, splenomegaly, hepatopathy, cholangitis, Cryptosporidium infection or inflammatory bowel disease and other clinical findings in lung function/chest CT/abdominal MRI as well as the current medical status. The clinical immunophenotype was subsequently analyzed and evalued as either remarkable or unremarkable regarding immunodeficiency.

3.2.1.3 Laboratory parameters

Cellular B- and T- cell immunology parameters, recommended according to Peron et al. (34) as part of an extended routine cellular immune status, were analysed using FACS, were also entered in the Excel table.
Collected B cell immunology parameters included:

- CD19 positive B cells (CD19/μl)
- B memory cells [i.e. non- class switched (CD19+CD27+IgD+), class switched (CD19+CD27+IgD-) and naive (CD19+CD27- IgD+) B cells]
- activated B cells (CD21lo CD38lo)
- transitional B cells (CD38hi IgMhi)
- class switched plasmablasts (CD38hi IgM-)

T cell immunology contained the following parameters:

- CD3 positive T cells (CD3+/μl)
- Helper T- cells (naive and activated) (CD3+CD4+)
- Cytotoxic T-cells (CD3+CD8+)
- Naive T cells (CD4+CD45RA+ (%of CD4)
- Gamma Delta T cell receptor (TCR)-positive T cells (TCRγδ+)
- Alpha Beta TCR CD4- and CD8-double-negative T cells (TCRαβ+CD4-CD8-CD56-)
- Natural Killer cells (NK/μl)

Furthermore Monocytes (Mono/μl) and stem cells in peripheral blood (stem cell in PB/μl) were measured.
FACS or Spectratyping was used to analyse the T cell receptor V-Beta repertoire. On the basis of sequence homology, TCR VB genes are classified as VB 1-24, according to the subfamily they belong to. VB10 and VB19, pseudogenes, are excluded.(146, 147) The analysis of the TCR Vbeta Repertoire in this thesis was focused on helper T-cells (CD4+) and cytotoxic T-cells (CD8+).

Serology parameters requested, representing a routine humoral immune status, included:

- an Immunoglobuline profile (IgG, IgG subclasses 1-4, IgM, IgA, IgD, IgE), immunofixation, electrophoresis
- autoimmune serology: ANA, anti dsDNA, Cardiolipin- IgG & IgM, b2GP- IgG & IgM
- Vaccination and serostatus: Diphteria, Tetanus, Varicella zoster virus, EBV, CMV, Hepatitis B, Morbilli, Rubeola, Hemophilus influenzae B, Pneumococci
- Coombs test (direct, indirect)

3.2.1.4 Genotype

To be included in this study, the diagnosis of CMMRD had to be proved genetically and was, as mentioned above, performed outside of the study as part of the routine diagnostic program at the Institute of Human Genetics, Medical University Innsbruck.
Information about the mutated gene (*MLH1, MSH2, MSH6* or *PMS2*), the mutation at cDNA level and the mutation at protein level was taken down if available.

3.2.1.5 Establishment of the database

Several excel tables containing all the variables described above where created with Microsoft Excel© Version 2016. The first table contained all the variables collected concening clinical information. The second table contained cellular B- an T- cell immunology parameters. The third table contained parameters concerning the T cell receptor repertoire and the last table contained all the serology parameters available. The database, composed of these same tables, was password protected and all patient data were anonymized.

3.3 Ethics votum

The ethics committee of the Medical University of Graz, registered at the Office for Human Research Protections (Institutional Review Board Registry number: IRB00002556), reviewed the study and approved the realisation of it without objections. (EK 29-178 ex 16/17)

3.4 Statistical analysis

Statistical analysis was made using two programms, namely Microsoft Excel© Version 2016 and Graph Pad Prism©.

Due to the small case number (n=11), the main approach of this statistical analysis was a thorough descriptive analysis of all the parameters collected in Excel tables.

Descriptive analysis of the data set was subsequently conducted in detail and used to note frequencies of occurrence of presenting conditions, mean values, standard deviations and median values using Microsoft Excel© Version 2016. Exploratory data analysis was used to sum up the main characteristics of our data set in tables and figures using both Microsoft Excel© Version 2016 and Graph Pad Prism©

4. Results

4.1 Patient collective & Main patient characteristics

The data of a total of 14 patients from 13 different families, that presented with a phenotype that indicated testing for CMMRD, were collected for this study. According to inclusion and exclusion criteria, three patients had to be excluded retrospectively (or were withdrawn from the physician in charge) because the diagnosis of CMMRD was not proven genetically. Three of the patients were presented to us aged above 18 (21, 26, 38) but were still inlcuded because of the genetic diagnosis of CMMRD. The mutation at cDNA level and mutation at protein level of one patient was not yet available at the time of cut-off, but this patient was still included. This resulted in a total cohort size of 11 CMMRD patients (n=11) from 11 different families, composed of 8 female (72,73%) and 3 male (27,27%) patients, from 8 different centers (Paris, Duisburg, Bratislava, Villejuif, Rambam/Haifa, Kassel, Madrid, Leiden) referred to as P1-P11 in this thesis. **Table 5** shows the main patient characteristics of all included patients. Age at point of inclusion into this study, sex, the genotype (respective mutated gene, mutation at cDNA level and mutation at protein level), the first symptom and the age at the occurence of the first symptom, as well as the clinical immunophenotype of each patient included are described in this table.

Table 5: Patient characteristic of all included patients (n=11)

Patient (Reference)	Age (y)*	Sex	Genotype (mutated gene, mutation at cDNA level, mutation at protein level)	First symptom/malignancy (age)	Known immunological alterations
P1 † (64)	38	f	*PMS2* c.137G>T p.Ser46Ile	Colorectal carcinoma (22)	none
P2	4	f	*MSH6* c.[467C>G]+[1316A>G] p.[Ser156Ter]+[Asp439Gly]	n.a.	none
P3 (148)	6	m	*MSH6* c.[3261dupC]+[3261dupC p.[Phe1088LeufsX5]+[Phe1088LeufsX5	CALMS, T-NHL** (2)	none
P4	8	m	*PMS2* c.ex12 c.2007-2A>G p.Val265_Gln314del	T-NHL (4)	none
P5	10	f	*PMS2*	Malignant Glioma (10)	none
P6 (38)	26	f	*PMS2* c.[2192T>G]+[2192T>G] p.[Leu731Ter]+[Leu731Ter]	Colorectal carcinoma (20)	n.a.
P7	21	f	*MLH1* c.[62C>A]+[2146G>A] p.[Ala21Glu]+[Val716Met]	T- NHL (1)	none
P8 †	1	f	*MLH1* c.332C>T p.(Ala111Val)	T-NHL (0,66)	none
P9	3	m	*PMS2* c.2007-2A>G	ALL (2)	n.a.
P10	7	f	*MSH6* c.[2653A>T]+[2653A>T] p.[Lys885Ter]+[Lys885Ter]	Wilms tumour (5)	n.a.
P11	7	f	*PMS2* c.[2444C>T]+[2444C>T] p.[Ser815Leu]+[Ser815Leu]	CALMS, no malignancy yet	none

**Age at point of inclusion into study, **T-NHL = first malignancy, P3 was tested for CMMRD before he developed his first malignancy, because of affected siblings and CALMS, †: Patient deceased*

The mean age of study participants at the time of inclusion into this study was 11,9 years and a median age of 7 was furthermore calculated. The youngest patient included was 1 year old (P8) and the oldest patient included was 38 years old (P1), which implies an age range of 37 years (1 – 38 years).

The first symptom of CMMRD noticed, which was a malignancy in 9 out of 10 patients (90%) where respective information was available, occured at a mean age

of 7,41 years (range 0,66-22 years). The youngest patient to develop a malignancy was P8, who developed a T-NHL at an age of 8 months. The oldest to first develop a malignancy was P1, who developed a colorectal carcinoma at an age of 22. 4 patients (P4, P7, P8, P9) first presented with a hematological malignancy, 3 of which were Lymphomas, more specifically T-NHL (P4, P7, P8). Two patients first presented with a Lynch associated GI maligancy, more specifically colorectal carcinoma (P1, P6), one patient first presented with a malignant brain tumour (P5). P3, a patient whose case has been published already by Ilencikova et al. in 2012 (148), was tested for CMMRD because of CALMS and 2 siblings suffering from CMMRD at the age of 1. At the age of 2 he developed a T-NHL, which represents the fifth hematological malignancy and fourth lymphoma as a first malignancy in this study population. P10 first presented with a Wilms tumour. P11 has not developed any malignancy yet, but was tested for CMMRD because of CALMS and parental consanguinity. **Figure 8** illustrates and gives an overview of the distribution of first symptoms, more specifically malignancies, in this study population.

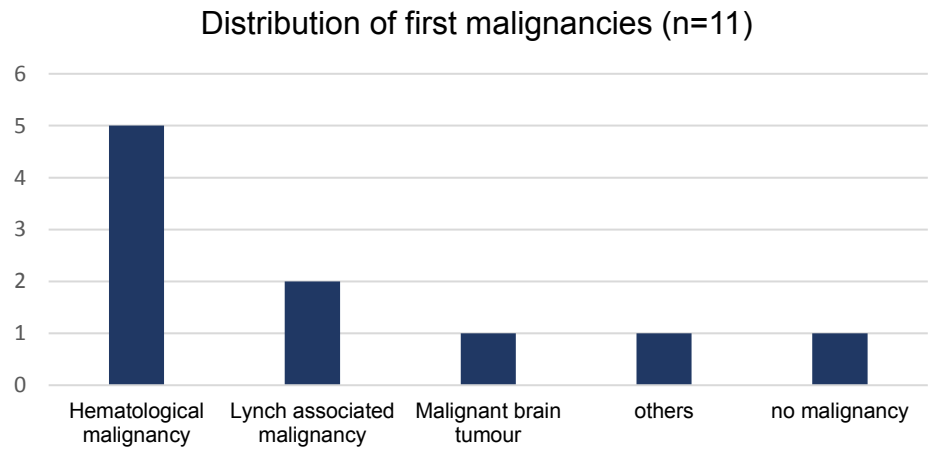

Figure 8: First malignancies -Distribution in the study population (n=11)

Hematological malignancies, described as first malignancy in 5 patients (45,45%), were found to be the most common first malignancy within the included 11 patients, followed by LS associated malignancies, described in 2 patients (18,18%), and finally malignant brain tumours, other tumours or no malignancy at all described in one patient each (respectively 9,09%). Out of the patients presented to us, the younger ones tended to present with hematological malignancies (P3, P4, P7, P8, P9). These group of 5 patients presented with a malignancy at a mean age of 1,93 years. The 2 patients who first presented with CRC (P1, P6) were 22 and 20 years

old. The patient who first presented with malignant glioma (P5) was 10 years old and the one who first presented with Nephroblastoma (Wilms tumour) was 5 years old.

Within the 11 included patients, biallelic mutations in 3 different MMR genes were detected as demonstrated in **Figure 9**.

6 patients (55 %) carried a homozygous *PMS2* mutation, 3 patients (27%) carried a biallelic *MSH6* mutations and 2 patients (18%) were found to carry a *MLH1* mutation in both alleles. No patient within the study population carried a homozygous *MSH2* mutation.

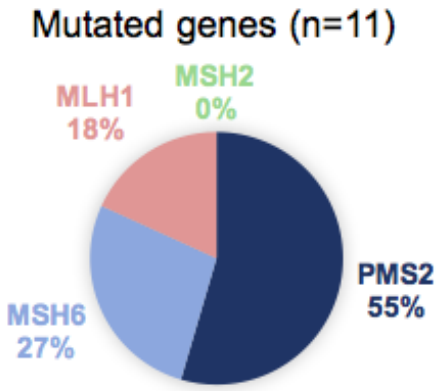

Figure 9: MMR genes mutated in the study population (n=11)

None of the included patients of whom data were available (n=8) was known to have any immunological alterations, e.g. known PID, prior to the inclusion into the study. Two of the patients included (P1, P8) are known to have deceased. P1 died in 2015 from a relapsed Glioblastoma and P8 died from refractory lymphoma in 2017 after suffering from early CNS and pleural relapse of this same malignancy.

4.2 Descriptive analysis of patient history

4.2.1 Family history, nonmalignant features and premalignancies

The clinical questionnaire included questions concerning family history, nonmalignant features and premalignancies. Collected data are detailed in **Table 6**. Revised Bethesda criteria for LS (149) were met in the family of P1, whose mother developed a CRC aged 46. The mother of P3 was presented to us as a possible CMMRD patient and was further diagnosed with LS. P3 also had affected siblings

as ascertained in Ilencikova et al. (148). P6 had a positive family history concerning malignancies. Her maternal grandfather suffered from CRC at the age of 40. All other family histories (72,73%), concerning LS or family tumours, evalued, were negative. Information concerning parental consanguinity was available in 9 patients and positive in 7 patients (77,78%) as shown in **Table 6**. 3 patients had a negative family history in terms of parental consanguinity. All patients (100%) included showed nonmalignant features. 3 patients (27,27%) were furthermore diagnosed with premalignancies. The nonmalignant feature observed the most were CALMS exhibited in 10 out of 11 patients (90,91%). Additionally, two more skin abnormalities were found in addition to CALMS. P2 showed Ash-leaf spots and a skin pigmentation disorder on the right calve was observed in P7. Other nonmalignant features observed were: Adenoma sebaceum and hepatic hemangioma in P1 and hemangioma in P2. P7 showed the biggest variety of nonmalignant features including, apart from skin abnormalities, cerebral cavernoma, which might be radiotherapy related, varicosis and vascular malformation. The only premalignancies observed were different types of adenomas (dysplastic adenoma of the colon, villous adenoma in the small intestine and multiple adenomas in both the small and the large bowel) in 3 patients (P1, P6, P7).

Table 6: Family history, Nonmalignant features & Premalignancies of all included patients (n=11)

	Family history			
Patient	**Family tumours**	**Parental consanguinity**	**Nonmaligant features**	**Premalignancies**
P1	LS family Mother: CRC (46y)	✓	Adenoma sebaceum Hepatic hemangioma	Dysplastic Adenomata (Colon)
P2	no	n.a.	Hemangioma CALMS Ash-leaf spots	
P3	Mother: LS affected siblings-CMMRD	✓	CALMS	
P4	no	no	CALMS	
P5	no	n.a.	CALMS	
P6	Maternal grandfather: CRC (40y)	✓	CALMS	Villous adenoma (small bowel)
P7	no	no	Cerebral cavernoma Varicosis Vascular malformation CALMS Skin pigmentation - disorder right calve	9 Adenomas (small, large bowel)
P8	no	✓	CALMS	
P9	no	✓	CALMS	
P10	no	✓	CALMS	
P11	no	✓	CALMS	

4.2.2 Malignancies

Malignancies occured in 10 out of 11 patients (90,91%). P 11, a *PMS2* mutation carrier, represented the only patient that has not yet developed any malignancy. Ten patients developed a total of 20 malignancies, premalignancies not taken into account. Malignancies found in the study population (n=11) were split into four groups: Hematological malignancies, Malignant brain tumours, LS associated malignancies and others. Out of 20 malignancies (100%), a total of 7 hematological malignancies (35%), 6 malignant brain tumours (30%), 5 Lynch associated tumours (25%) and 2 others, a malignant Phylloides tumour and a Wilms tumour (10%), were found in the 10 patients who developed malignancies.

The mean age of malignancy onset was 7,4 years (0,66-22 years). The mean age of malignancy onset in homozygous *PMS2* carriers (n=5) was 11,6 years. Malignancies in the two *MSH6* carriers were first developed aged 2 and 5 and *MLH1* carriers were the youngest with an age of 1 and 0,66 years at malignancy onset. **Table 7** gives an overview of the occurence of malignancies in all patients and, if information was available, the respective age the malignancy was developed.

Table 7: Malignancies: Occurence of malignancies in all included patients

		Malignancy (age in years at diagnosis)			
Patient	**Mutation**	**Hematological**	**Brain**	**LS associated**	**Others**
P1	*PMS2*	-	Glioblastoma (34 y)	CRC (22 y) Duodenal Ca (36 y) Endometrial Ca (36y)	
P2	*MSH6*	-	Anaplastic Medulloblastoma (age not reported)	-	-
P3	*MSH6*	T-NHL (2y) ALL (6y)	-	-	-
P4	*PMS2*	T- NHL (4y)	-	-	-
P5	*PMS2*	-	Malignant Glioma (10y)	-	-
P6	*PMS2*	-	Low grade diffuse astrocytoma (23y) → High grade Glioma (26y)∗	CRC (20y) Papilla Vateri Ca (22y)	-
P7	*MLH1*	T-NHL (1y) B-NHL (15y)	Glioblastoma (21y)	-	Malignant Phylloides tumour (16y)
P8	*MLH1*	T-NHL (8m)	-	-	-
P9	*PMS2*	ALL (2y)	Glioblastoma (3y)	-	-
P10	*MSH6*	-	-	-	Wilms tumour (5y)
P11	*PMS2*	-	-	-	-

(∗ was counted as one malignancy)

The 10 patients who developed a total of 20 malignancies showed the full spectrum of malignomas typical for this CPS (36) as shown in **Figure 10**. High grade gliomas, diagnosed 5 times (25%), represented the most common malignancy in this study population, closely followed by T-NHL, which was found in 4 patients (20%). Furthermore 2 ALL, 2 CRC and 2 Duodenal/Papilla vateri Carcinomas, which respectively represented 10% of malignanciess diagnosed in total each, were diagnosed. All other malignancies (B-NHL, Medulloblastoma, Endometrium Carcinoma, Wilms tumour, Phylloides tumour) were only diagnosed once (5% each). **Figure 10** shows the distribution of malignancies diagnosed. As seen in this same figure, each group of malignancies was represented in this study population and apart from the small number of CRC, reflects previous suggestions of distribution (39).

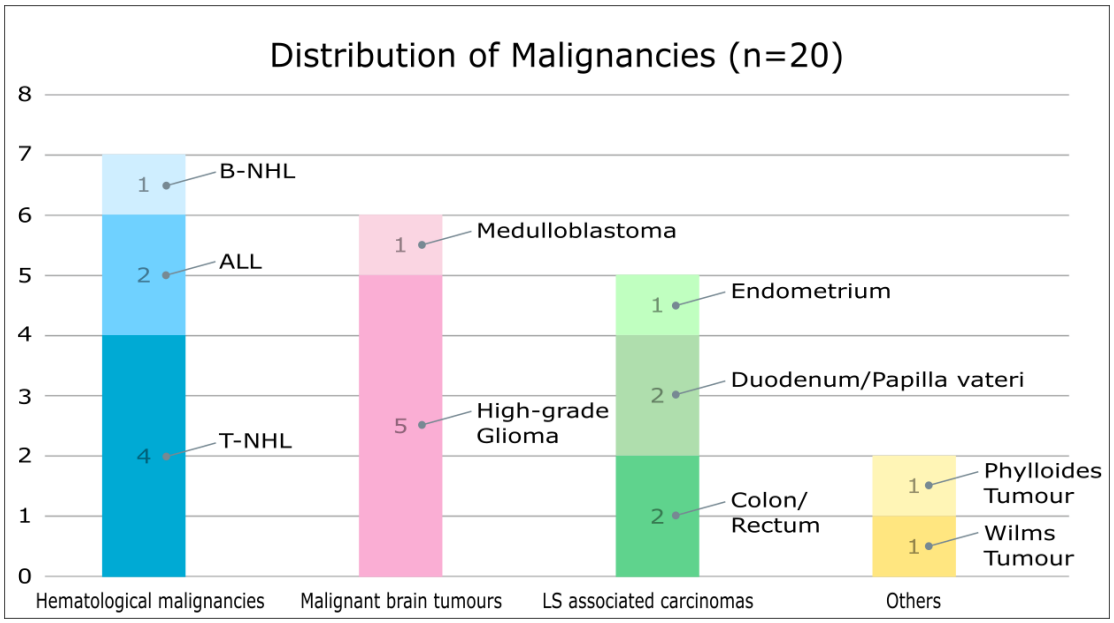

Figure 10: Distribution of malignancies(n=20) diagnosed in 10 out of 11 included CMMRD patients.

Hematological malignancies were diagnosed at a mean age of 4,38 years (range 0,66- 15 years), which was the earliest type of malignancy to be diagnosed in included patients. The mean age of diagnosis in the group of malignant brain tumours was 18,2 years (range 3-34years). LS associated carcinomas developed the latest in this study population with a mean age of 27,5 years at diagnosis (range 20-36 years). It ought to be mentioned though, that only the 19 malignancies, where information about the respective age they were diagnosed at was available, were considered in this calculation as illustrated in **Figure 11**. Out of the 10 patients who

developed malignancies, 8 (80%) were diagnosed with their first malignancy in childhood or adolescence (<18 years).

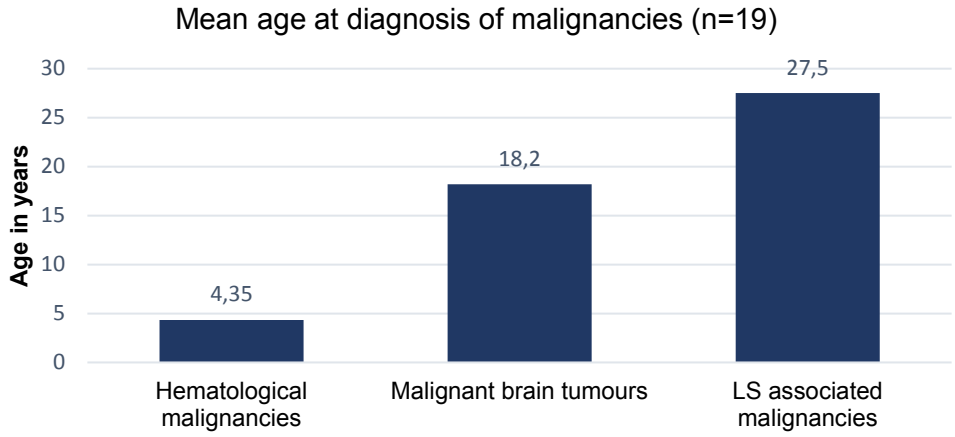

Figure 11: Mean age at diagnosis of malignancies (n=19) in 10 out of 11 included CMMRD patients.

LS-associated malignancies where only observed in *PMS2* mutation carriers (P1, P6). Malignant brain tumours developed in four *PMS2* mutation carriers (P1, P5, P6, P9), one *MSH6* mutation carrier (P2) and one *MLH1* mutation carrier (P7). Two *PMS2* mutation carriers (P4, P9), 1 *MSH6* mutation carrier (P3) and all included *MLH1* carriers (P7, P8) revealed hematological malignancies in their history.

Out of the 10 patients that developed malignancies, 5 patients (50%) developed more than one malignancy. Three of the five patients with more than one malignancy in their history carried a *PMS2* mutation (P1, P6, P9), one patient carried a *MSH6* mutation (P3) and one patient carried a *MLH1* mutation (P7).

4.2.3 Oncological treatment

Information concerning oncological treatment could be obtained in 6 patients. Chemoptherapy was administered in P1, P3, P4, P6, P7 and P8 (100%). Temozolamid was used in P1 and P6 for glioblastoma treatment. P1, P6 and P7 were previously treated with radiotherapy (50%) for glioblastoma as well. P1 (GI: surgical resection CRC, endoscopic resection Duodenal Ca, Endometrial Ca: surgical resection) and P6 (GI: total colectomy, Whipple glioma: surgical resection) underwent surgical treatment (33,33%). However, detailed information concerning time distance between blood tests and therapy as well as the current therapy status was not obtainable.

4.3 Immunophenotype

4.3.1 Clinical Immunophenotype

The evaluation of the clinical immunophenotype of included patients was based on a clinical questionnaire which was send to and filled out by respective physicians in charge. Out of 11 patients included, 8 completed questionnaires (72,73%) could be used for evaluation. No clinical questionnaire was available of P6, P9 and P10. P6 was no longer cooperating and incompliant with her follow- up. Questionnaires of patients P9 and P10 were not received before cut-off.

The questionnaire was aimed at inquiring immunodeficiency warning signs, infections and signs of immune dysregulation of the respective patient. After evaluating the questionnaires, the clinical immunophenotype of each patient was interpreted. The results of this analysis are detailed in **Table 8**.

None of the 8 patients evaluated had a history of family immunodeficiency. Two patients (P3, P8), 25%, were previously hospitalized for infections, although P8 was only hospitalized for infections during chemotherapy. Reactions or complications after life vaccines, if they were administered, or other immunodeficiency warning signs, i.e. pathological healing of the navel and delayed growth, respectively reduced thriving, were not observed in any of the patients. The frequency of infections per year was referred to as normal in four patients (50%), P4, P5, P7 and P11, by their respective physicians in charge. P1 was described to not have any infections per year (0), P2 had an estimated 7 infections per year and 4-5 infections per year occurred in P3. Overall these numbers were interpretated as unremarkable and within normal range, which means that a total of 7 out of 8 patients (87,5%) showed no increased frequency of infections.

P8, who was also previously hospitalized for infections, was furthermore described to show an increased number of infections since chemotherapy started, meaning that both hospitalization for infections as well as the increased frequency of infections that was observed were most likely related to chemotherapy. The severity of infections that were observed in patients evaluated was split into groups, namely simple viral, simple bacterial, complicated viral and complicated bacterial as shown in **Figure 12**.

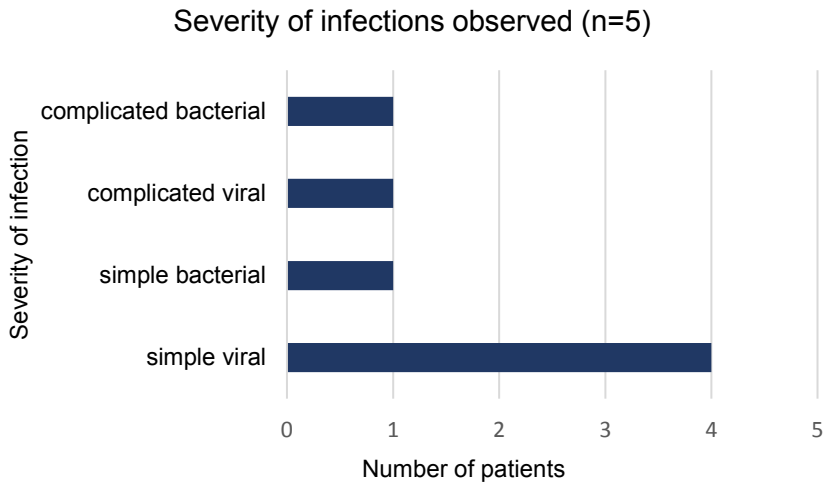

Figure 12: Severity of infections observed in CMMRD patients (n=5).

Five patients were described to have suffered from infections. Simple viral infections were described in four patients (50%) and therefore the most and were either located in the ear-nose-throat tract (ENT), as described in three patients (P3, P5, P7), or the respiratory tract as described in one patient (P2). Simple bacterial infections, affecting the lungs, were only observed in P3. P8 was the only one to show both complicated viral and complicated bacterial infections. In her case, they presented as lung infections and bacteremia. Infectious agents were tested in three patients. P3, who presented with simple viral and simple bacterial infections, was diagnosed with Staphylococcus aureus, MRSA and Aspergillus fumigatus infections during chemotherapy. P7, who was only described to be affected by simple viral infections to date, showed a normal spectrum of infectious agents. P8, who presented with both complicated viral as well as bacterial infections, was found to be affected by opportunistic agents. The response to antibiotic therapy, which was also considered in this questionnaire, was decribed to be normal in three patients (37,5%). Two patients, P5 and P11, (25%), never needed antibiotics to date and the response to these is therefore unknown. A delayed response to antibiotics was observed in P3 and P8 (25%). No information concerning response to antibiotic therapy was available in P1. Corticosteroids or immunosuppression were needed in P1, P3 and P8 (37,5%). Physicians in charge of P1 and P3 clearly stated the usage of these agents as in context of oncological treatment. Five patients (62,5%) never required corticosteroids or immunosuppression. In terms of autoimmunity or immune dysregulation, nothing remarkable, apart from mild axillary

lymphoproliferation in P3, was observed and as shown in **Table 8**, all other autoimmunity related questions were answered with no.

To summarize, the overall clinical immunophenotype was not found to be remarkable in any of the patients. P3 (biallelic MSH6 mutation carrier) and especially P8 (MLH1 mutation carrier) were found to be the most remarkable patients considering their hospitalizations for infections, the frequency and severity of infections in P8, the infectious agents described in both patients, as well as their delayed response to antibiotics and the requirement for corticosteroids and immunosuppression. As these observations and events however were only described after or during chemotherapy, they have to be considered as secondary immunodeficiency and do not allow to conclude that a primary immunodeficiency due to CMMRD existed.

Table 8: Clinical immunophenotype of included CMMRD patients (n=8)

Clinical characteristics	P1	P2	P3	P4	P5	P7	P8	P11
Immunodeficiency warning signs								
Family immunodeficiency	no	no	no	no	no	no	no	no
Hospitalization for infections	no	no	yes	no	no	no	during chemo	no
Reactions/complications after life vaccines	no	no	no	no	no	no	non administered	no
Pathological healing of the navel	no	no	no	no	no	no	no	no
Delayed growth during childhood/reduced thriving	no	no	no	no	no	no	no	no
Infections								
Frequency (per year)	0	7	4-5	normal	normal	normal	increased since chemo	normal
Severity	n.a.	simple viral	simple viral simple bacterial	n.a.	simple viral	simple viral	complicated viral complicated bacterial	n.a.
Localisation	n.a.	respiratory tract	ENT, bacterial: lungs	n.a.	ENT	ENT	lungs bacteremia	n.a.
Infectious agents	n.a.	n.d.	during chemo: Staphylococcus aureus, MRSA, Aspergillus fumigatus	n.a.	n.d.	normal spectrum	opportunistic	n.a.
Response to antibiotics	n.a.	normal	delayed	normal	never needed	normal	delayed	never needed
Requirement of corticosteroids or Immunosuppression	yes,oncological	no	yes, during chemo	no	no	no	yes	no
Autoimmunity/Immune dysregulation								
Granuloma	no	no	no	no	no	no	no	no
Autoimmunity	no	no	no	no	no	no	no	no
Autoinflammation/recurrent fever	no	no	no	no	no	no	no	no
Lymphoproliferation/Splenomegaly	no	no	yes, mild axillary	no	no	no	no	no
Hepatopathy, Cholangitis, Cryptosporidium infection	no	no	no	no	no	no	no	no
Inflammatory bowel disease	no	no	no	no	no	no	no	no
Interpretation regarding Immunodeficiency	**unremarkable**	**unremarkable**	**unremarkable**	**unremarkable**	**unremarkable**	**unremarkable**	**unremarkable**	**unremarkable**

4.3.2 Cellular / humoral immune phenotype

As part of the analysis of the cellular/ humoral immune phenotype of CMMRD patients, FACS analyzed parameters (B- and T- cell subpopulations), the T cell receptore repertoire of all included patients (n=11) and an Ig level assay were evalued and subsequently interpretated regarding immunodeficiency.

4.3.2.1 B- and T-cell immunology /Immune status

4.3.2.1.1 B- cell subsets and differentiation

Determination of B cell subsets, especially in immunodeficient patients, has been subject of research in the past few years (112, 150). Thus, FACS-analyzed parameters of B cell subsets of included patients regarding B cell development were further looked at and are detailed in **Table 9**. Evaluable data on B cell subsets were only available for 10 patients, as B cell subsets could not be detected in P9 and this patient was thus not evalued.

As an evident increase of memory B-cells and decrease of naive B cells with age is established, age related reference values (5^{th} to 95^{th} percentile) of healthy individuals of five age groups were taken from two studies for these subsets (112, 150) and are listed below our patient's values. Reference values for other B cell subsets were not adjusted to five different age groups.

As shown in **Figure 13**, 6 out of 10 patients presented with a reduced absolute number of CD19+ B-cells or B-lymphopenia.

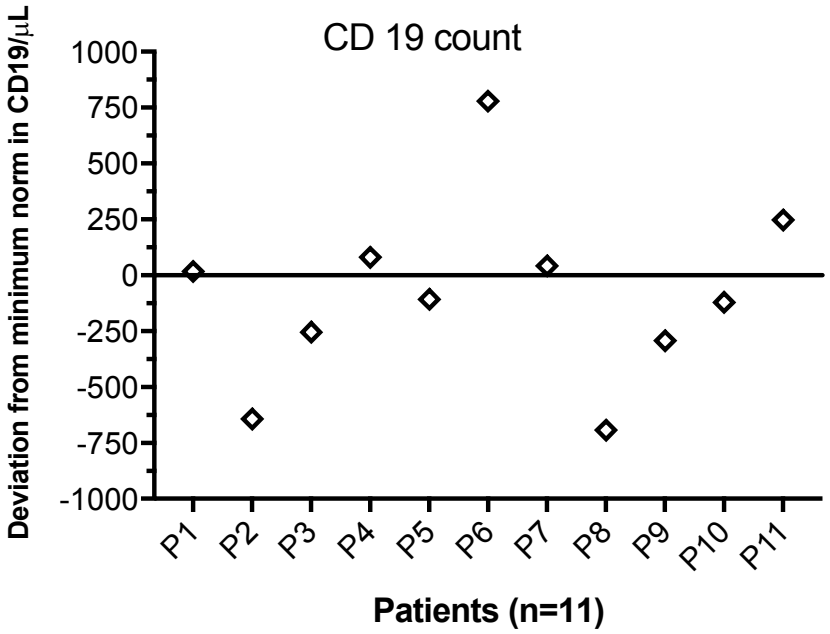

Figure 13: CD19 count: Deviations from age-specific lower limits of CD19/µl, *The additional line at zero represents the minimum level of the respective age specific normal range of CD19/µl, values underneath the line are reduced levels, values above the line are within normal range or increased (P6, P11)*

Severe reduction of number circulating B-cells was detected in P2, P3, P8 and P9 and a moderate reduction was found in P5 and P10.

The memory B cell department (CD27+), csBm and ncsBm, was evalued in 10 patients.

Figure 14 displays the calculated deviation from the respective age dependent minimum level of a normal percentage of csBm and ncsBm (line at zero represents the minimum age dependent norm). All values above the line were within normal range or increased (P3).

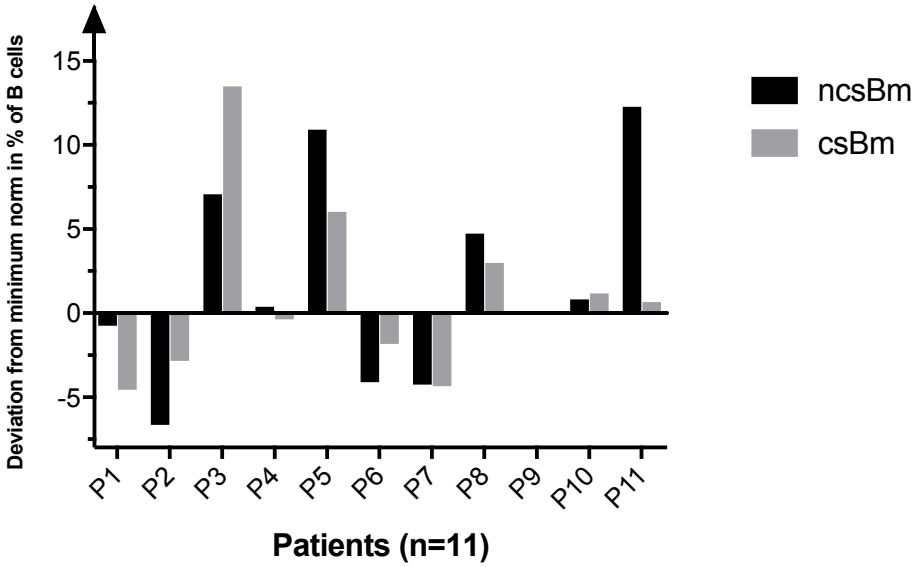

Figure 14: CsBm and NcsBm cells in CMMRD patients: Deviations from age dependent minimum norm.*The additional line at zero represents the age dependent minum norm of csBM in percentage of B-cells. Values above this line are normal or increased (P3)*

A reduced proportion of csBm cells was exhibited in five patients (P1, P2, P4, P6, P7) ,50%, as measured in percentage of total B cells with values clearly below the 5th percentile in all patients but P4 who showed just a slightly decreased percentage of csBm. A severe reduction of switched memory cells according to EUROclass (151) (<2 % of total B- cells) could be detected in P1 and P2 (20%). NcsBm were decreased in 4 patients (P1, P2, P6, P7) or 40%.

Table 9: B cell subsets and age related reference values

Cellular immune system	CD19/µL	CD19+CD27+IgD+ (ncsBm) %B	CD19+CD27+IgD- (csBm) %B	CD19+CD27-IgD+ (naive) %B	CD21loCD38lo (activ) %B	CD38hi IgMhi (trans) %B	CD38hi IgM- (csPlasmabl)%B
B- cell subsets P1-P11							
P1 (38y)	317	**6,75**	**1,93**	**89,29**	**84,21**	**0,73**	**0,1**
P2 (4y)	**57**	**0.35**	**1,06**	**93,47**	**80.91**	5,78	**0,35**
P3 (6y)	**46,5**	10	**30**	**0**	6,67	**0**	**0**
P4 (8y)	381	3,32	**3,47**	**88,12**	**40,33**	2,55	**0,15**
P5 (10y)	**193**	13,85	9,88	74,75	6,84	8,22	**0**
P6 (26y)	**1079**	**3,3**	**4,67**	**84,29**	**32,54**	1,39	**0**
P7 (21y)	342	**3,15**	**2,14**	**92,24**	62,95	1,29	**0,18**
P8 (1y)	**8**	8	4	**80**	**0**	**47,62**	**0**
P9 (3y)	**7**	/	/	/	/	/	/
P10 (7y)	**179**	3,21	5,02	**90,54**	5,14	20,09	0,65
P11 (7y)	548	15,21	4,52	73,87	8,29	5,6	**0,15**
Controls/Reference values 5th-95th percentile							
1 year ∗(n=26)	700-1300	3,25-10,75	1-5	83,25-93,75	1-11	1-25	0,4-3,6
2-3 years ∗(n=38)	700-1300	4,9-14,2	2,9-9,2	74,7-90,5	1-11	1-25	0,4-3,6
4-5 years ∗(n=38)	700-1300	7-15,2	3,9-16,2	69,9-85,6	1,11	1-25	0,4-3,6
6-10 years ∗(n=38)	300-500	2,93-19	3,85-16,5	63,1-89,15	1-11	1-25	0,4-3,6
19-61 years ∗(n=54)	300-500	7,4-32,5	6,5-29,1	42,6-82,3	1-11	1-25	0,4-3,6

∗reference values for ncsBm, csBm, naïve B-cells were taken from Huck et al. 2009 (112) for the age groups 1 year, 2-3 years, 4-5 years, 6-10 years and from Warnatz and Schlesier 2008 (150) for the age group 19-61 years and csPlasmablasts, n in brackets refers to the number of patients tested in the study for these same reference values, , moderately remarkable deviating results are printed in black boldface, strongly/severely remarkable deviating results are printed in red boldface, remarkable increased values are printed in green boldface

Naive B- cells were clearly increased in five patients (P1, P2, P4, P6, P7), negative in P3 and slightly decreased in P8. Transitional B cells, also belonging to the CD27 negative naïve population of B cells (150), were clearly expanded in P8, slightly decreased in P1 and negative in P3. CD21low activated B cells, also part of the CD27 negative family, were expanded in P1, P2, P4 and P6 and negative in P8. Relatively low or negative plasmablast levels, as part of the CD27 positive so-called "memory gate" (150), were exhibited in all patients but P10.

4.3.2.1.2 T- cell subsets

T-cell subsets, analyzed with FACS, were available for all 11 patients (n=11) and are detailed in **Table 10**. Results deviating from the norm shall be discussed in this chapter.

FACS analysis revealed T-lymphopenia, a decreased CD3+/μl count, in six patients (54,55%), one of which could be interpreted as severe (P8). Helper T cells (CD3+CD4+) were reduced in five patients (45,45%). CD4+lymphopenia was moderate in P2 and P10 and severe in P3 and P8. The cytotoxic T cell count (CD3+CD8+) was severly depressed in P8 again. CD4/CD8 ratio was calculated and found to be inverted in P3, P4 and P10 and elevated in P8. No reduction of naïve T cells (CD4+CD45RA+ (%of CD4) was detected in this study population. Gamma Delta T cells (TCRgd+) were strongly elevated in P3. Natural killer cells, reduced in 5 patients (45,45%), were found to be moderately reduced in P2, P5 and P10. A severe reduction of natural killer cells was detected in P8. Monocytes were found to be reduced in 4 patients (36,36%) in this study population and moderately increased P6 and P9 (18,18%). Stem cells in peripheral blood were detected in six patients (see Table 9), whereas especially the stem cell count detected in P6 and P9 was interpretated as remarkable. This could indicate an overshooting reaction after aplasia. However, no sufficient information about their current therapy and status was available.

Table 10: Cellular immune system: T cell subsets and small immune status of all patients included (n=11)

Cellular immune system	range	P1	P3	P4	P5	P6	P7	P10	P11	range	P2	P8	P9	Mean value/ SD
T – cell subsets														
CD3+ /µL	1400-2000	2053	**833,00**	2479	954	2481	1378	1123	**5139**	1800-3000	**644**	**71**	1839	1726,73 SD: 1363,60
CD3+CD4+	700-1100	1342	**266,00**	939	543	1485	776	**405**	**3209**	1000-1800	**483**	**53**	1088	962,64 SD:828,13
CD3+CD8+	600-900	639	**556,00**	1278	377,00	948	538	681	**1748**	800-1500	**189**	**15**	613	689,27 SD: 465,73
CD4+CD45RA+ (%of CD4)	>11-20-30% (age-dep.)	21,75	32,79	44,72	77,22	n.d.	19,91	36,30	53,18	>11-20-30% (age-dep.)	60,8	39,93	36,64	42,32 SD: 16,65
TCRab+CD4-CD8-CD56- (%CD3)	<2% of CD3	n.d.	0,00	0,04	0,20	n.d.	0,21	0,20	0,06	<2% of CD3	0,15	0	0,09	0,11 SD: 0,08
TCRgd+ (% von CD3)	<11-15 % of CD3	1,7	**26,21**	15,3	5,93	n.d.	3,41	7,10	7,21	<11-15%of CD3	3,29	5,41	3,17	7,87 SD: 7,10
NK /µL	200-300	266	276	271	**140,00**	189	323	**120**	566	200-300	**124**	**24**	252	231,91 SD: 135,73
Mono /µL	400-1000	320	398,00	137	400,00	**1664**	690	427	609	400-1000	735	374	**2254**	728 SD: 615,60
stem cells in PB /µL		n.d.	n.d.	0	1,00	**14**	1	1	3		0	2	**13**	3,89 SD: 5,22
Interpretation regarding Immunodeficiency (+ B-cell subsets)		borderline B reduction severe csBm-reduction activated B increased	B lymphopenia Cd4T Lymphopenia gdT high	csBm reduced activated B increased	B lymphopenia mild T lymphopenia	csBm reduced activated B increased	csBm reduced activated B increased mild T lymphopenia	B lymphopenia mild T lymphopenia			CVID like severe csBm reduction B lymphopenia activated B increased moderate Panlymphopenia	Severe Panlymphopenia	B lymphopenia	

SD= standard deviation, moderately remarkable deviating results are printed in black boldface, strongly/severely remarkable deviating results are printed in red boldface, remarkable increased values are printed in green boldface, note: not all deviations from normal range are printed in boldface

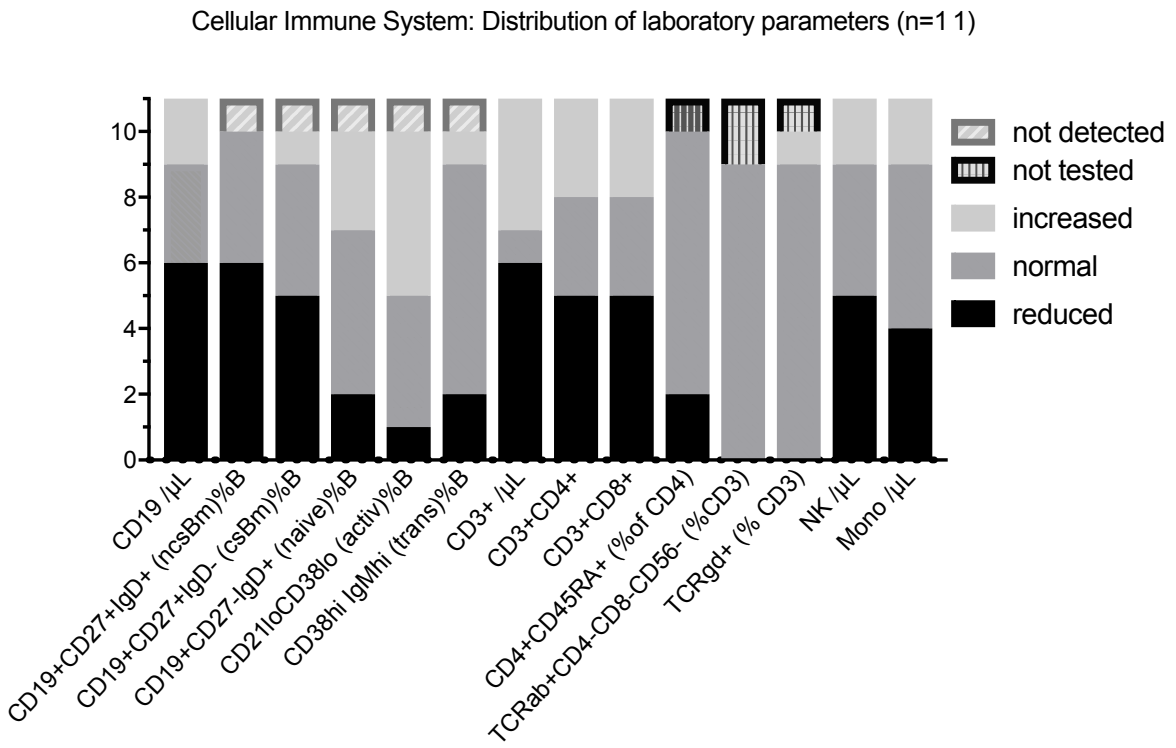

Figure 15: Cellular immune system: Distribution of laboratory parameters of all CMMRD patients included (n=11)

Figure 15 displays the distribution of laboratory parameters in all patients, split into groups namely: reduced, normal, increased, not detectable and not tested and gives an overview of how many values where overall deviating from normal range.

4.3.2.2 T cell receptor repertoire

The T cell receptor -Vbeta repertoire of a total of 11 patients (n=11) was evalued and analyzed to get an idea of the whole immunophenotype. The TCR repertoire of 4 patients (P2, P3, P4, P6) was evalued using FACS, the TCR repertoire of the remaining 6 patients (P1, P5, P7, P8, P9, P10, P11) was evauled using spectratyping (RNA). This analysis was emphasized on evaluating the respective TCR Vbeta repertoire of helper T- cells (CD4+) and cytotoxic T-cells (CD8+).

The TCR-Vbeta repertoire was found to be normal in all categories evalued in 6 out of 11 patients (54,55%), exhibiting polyclonal patterns with Gaussian distribution. The repertoire in CD4+ T cells was mildly reduced in 4 patients (36,36%) and severly reduced in 1 patient (9,09%). CD8+ T cells revealed a mild reduction in 3 patients (27,27%), a moderate reduction in 1 patient (9,09%) and a severe reduction of the TCR- Vbeta repertoire in 1 patient (9,09%) as well.

P8 showed an oligoclonal and skewed TCR repertoire in both CD4+ and CD8+ T-cells.

Overall, P8, was found to be the only patient to show restricted TCR diversity patterns.

Table 11: T cell repertoire of all patients included (n=11)

	TCR-Vbeta repertoire (24 bands)	CD3	CD3+CD4+	CD3+CD8+
P1	RNA		mildly reduced	moderately reduced
P2	FACS	normal	normal	normal
P3	FACS	normal	normal	normal
P4	FACS	normal	normal	normal
P5	RNA		normal	normal
P6	FACS	normal	normal	normal
P7	RNA		mildly reduced	mildly reduced
P8	RNA		severly reduced	severly reduced
P9	RNA		mildly reduced	mildly reduced
P10	RNA		normal	normal
P11	RNA		mildly reduced	mildly reduced

severity of reduction of T cell repertoire is color coded (mildly reduced= yellow, moderately reduced= orange, severly reduced= red, normal =green)

4.3.2.3 Immunoglobuline profile/ serum Ig concentrations assay

The immunoglobuline profile, including total IgG, IgA, IgM and IgE, of a total of 10 patients (n=10) was analyzed. A serum Ig level assay of P4 was not available.

Table 12 displays the immunoglobuline profile, composed of total IgG, IgA, IgM and IgE, of all patients evalued. As serum immunoglobuline levels are age dependent and differ in children of different age (152), the respective age dependent reference values, referred to as range, are respectively pointed out next to each parameter obtained.

Table 12: Immunoglobuline profile of included patients (n=10)

	IgG (g/L)	range	IgA (g/L)	range	IgM (g/L)	range	IgE (IU/ml)	range
P1 (38)y	7,5	6,8-12,7	2,13	0,84-2,69	2,74	0,74-2,09	4	<100
P2 (4y)	6,2	5,4-13,4	neg.	0,38-2,2	3,68	0,45-1,69	51	<100
P3 (6y)	9,4	5,9-14,3	0,61	0,38-2,2	0,61	0,45-1,69	14,3	<100
P5 (10y)	3,02	7,0-16,0	0,37	0,7-4,0	1,22	0,4-2,3	n.t.	
P6 (26y)	9,14	7,0-16,0	3,34	0,7-4,0	2,12	0,4-2,3	n.t.	
P7 (21y)	3,73	7,0-16,0	1,56	0,82-4,53	0,42	0,46-2,3	<15	<100
P8 (1y)	0,68	2,86-15,6	0,13	0,19-1,75	0,36	0,43-1,63	n.t.	
P9 (3y)	1,06	4,0-11,0	0,24	0,1-1,6	0,17	0,5-1,8	n.t.	
P10 (7y)	9,04	6,0-12,3	0,93	0,3-2,0	1,22	0,5-2,0	n.t.	
P11 (7y)	11,9	6,7-15,3	1,2	0,7-4,0	1,3	0,4-2,3	3	<100

color code: reduced parameters: red, normal parameters: green, increased parameters: yellow, n.t.=not tested

As shown in Table 12 above, the serum Ig concentrations (total IgG, IgM, IgA and IgE) showed abnormalities in more than half of the patients. Hypogammaglobulinemia was observed in four out of 10 patients (40%), namely P5, P7, P8 and P9. Total serum IgG was reduced (relative to the normal reference values) in four patients (40%). It was found to be strongly depressed in P5, P7, P8 and P9. In addition to the depressed serum IgG levels, IgA was reduced in 20% or 2 patients (P5, P8). IgA was selectively negative in one patient (P2) out of 10 (10%). IgM was found to be reduced in three patients (P7, P8, P9) or 30%. All IgM and/or IgA reductions detected, apart from the IgA deficiency P2 showed, were thus

combinated with serum IgG levels below the respective reference range. Two patients (20%) evalued showed elevated levels of IgM (P1, P2), whereas the elevation was stronger in P2 with an elevation of 108% above the maximum normal level. Those were found to be combinated with normal levels of IgG and negative IgA in P2. P1 did not show any abnormalities in neither IgG nor IgA.

As P2 revealed negative IgA and elevated IgM, the normal IgG level might be explainable by IgG substitution, albeit this information was not obtainable in retrospective. P5 showed decreased levels of IgG and IgA and normal levels of IgM which fits the serological definition of HIGM (153).

P3, P6, P10 and P11 showed normal levels of total IgG, IgA and IgM. IgE was tested in five patients only (P1, P2, P3, P7, P11) and found to be unremarkable in all.

The distribution of immunoglobuline parameters in patients included is shown in **Figure 16**.

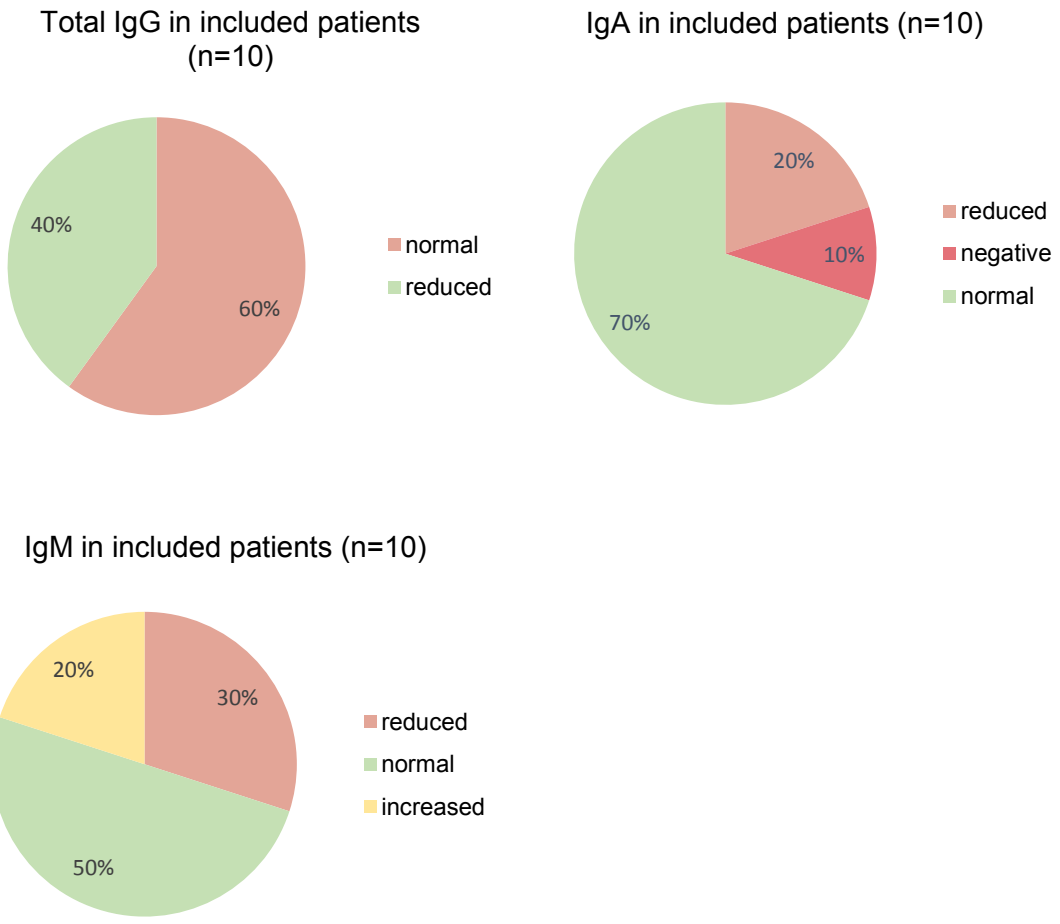

Figure 16: Immunoglobuline profile: IgG, IgA and IgM of included CMMRD patients (n=10).

Figure 17 illustrates deviations from age specific lower limits (IgG, IgA) and since there are suggestions for CMMRD patients to show signs resembling HIGM (34, 123)- upper limits (IgM) of serum Ig levels as depicted in **Figure 18**.

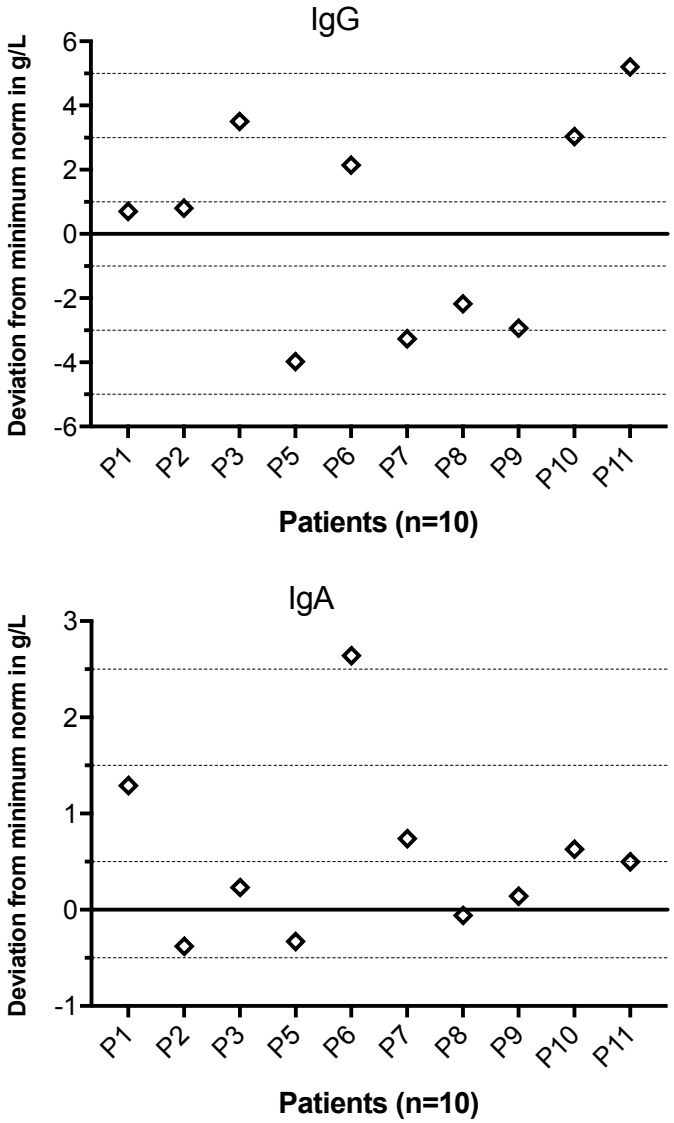

Figure 17: IgG, IgA: Deviations from age specific lower limits of serum Ig levels*, The additional line at zero represents the minimum level of the respective age specific normal range, values underneath the line are reduced levels, values above the line are within normal range*

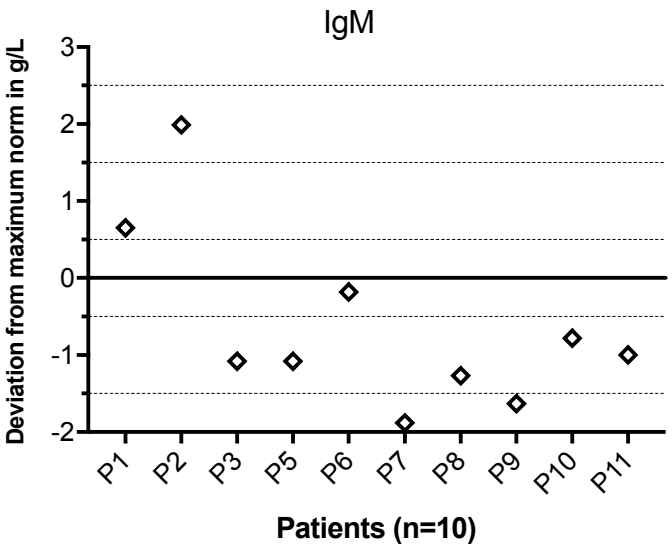

Figure 18: IgM: Deviations from age specific upper limits of serum IgM levels*. The additional line at zero represents the maximum level of the respective age specific normal range, values above the line are elevated levels, values underneath the line are within normal range or reduced (P7, P8, P9)*

4.3.2.3.1 IgG subclasses

In addition, IgG subclasses of a total of five patients were obtained (n=5), as shown in **Table 13**. As already described for total IgG, IgA and IgM, IgG subclasses levels are age related values in children, that differ from age group to age group and that have been subject of studies for a long time.(154-157)

The respective age dependent reference range was therefore put in brackets next to the evalued parameters and referred to as “range”.

Table 13: IgG subclasses of patients evalued (n=5)

	P1 (range)	P2 (range)	P3 (range)	P7 (range)	P11 (range)
Total IgG (g/L)	7,5 (6,8-12,7)	6,2 (5,4-13,4)	9,4 (5,9-14,3)	**3,73** (7,0-16,0)	11,9 (6,7-15,3)
IgG1	**3,5** (3,8-9,3)	4,9 (3,0-8,4)	4,17 (3,5-9,1)	3,4 (2,8-8,0)	8,7 (3,7-9,3)
IgG2	3,2 (2,4-7,0)	**0,66** (0,7-2,55)	0,86 (0,85-3,3)	**0,64** (1,15-5,7)	1,69 (1,0-4,0)
IgG3	1,15 (0,2-1,7)	0,6 (0,17-0,97)	1,18 (0,20,1,04)	0,75 (0,24-1,25)	0,82 (0,22-1,09)
IgG4	**0,003** (0,04-0,86)	0,066 (0,017-1,16)	**0,006** (0,03-1,577)	**<0,01** (0,052-1,250)	**<0,01** (0,043-1,90)

abnormal values are printed in boldface

The IgG subclass serum assay revealed mild abnormalities in all patients, one of which also showed a decreased serum level of total IgG (P7).

P2 displayed a total IgG that was normal, but combined with IgG2 deficiency.

IgG2 deficiency was furthermore detected in one more patient (P7), meaning that 2 out of five patients tested (40%) revealed this specific deficiency. P7 displayed a strongly decreased total IgG and, like already mentioned, IgG2 deficiency, but also IgG4 deficiency. Low levels of IgG4 were detected in 4 (P1, P3, P7, P11) out of 5 patients tested (80%).
Decreased IgG1 was only found in P1. No patient evalued showed decreased levels of IgG3.
It ought to be kept in mind though, that it is not seldom, that IgG4 levels are not detectable in children. They decrease after birth and reach their lowest level around 7 to 12 months and then start to increase in children as they get older and reach their so called “plateau “ at about 12-14 years (154).

To recapitulate, P1 showed a high serum level of IgM combined with decreased IgG1 and IgG4. P2 was found to show IgA deficiency, high IgM and a low IgG2, but normal levels of total IgG and questionable IgG substitution. P3 revealed an unremarkable immunoglobuline profile, apart from IgG 4 deficiency. P5 showed low levels of both total IgG and IgA in combination with normal IgM resembling HIGM. No abnormalities were found in P6. Decreased levels of IgG (in combination with IgG2 and IgG4 deficiency) and IgM were detected in P7. P8 presented with low levels of IgG, IgA and IgM. Reduced total IgG combined with reduced IgM was found in P9. P10 was not found to show any abnormalities. P11 presented with selective IgG4 deficiency. Mild abnormalities in the serum Ig level assay were therefore detected in 8 out of 10 patients (80%). P10 was, however, not tested for IgG subclasses.

4.3.3 Serology results

4.3.3.1 Vaccination/Serostatus

The requested vaccination status and serostatus (EBV, CMV) of a total of seven patients (n=7) was partially evalued and summarized in **Table 14**. The full vaccination and serostatus was, however, as a drawback of this study not available in any of the patients included. The fact, that no information about the actual vaccination status (performed administration of vaccines) of included patients was available, impaired analysis of these same parameters. Negative results, that is to say, can either be interpretated as no vaccination received or not enough immunity

build, with the latter being reffered to as incomplete immunization with no or no sufficient protection given (158).

Table 14: Vaccination and Serostatus of patients evalued (n=6)

Parameter (reference)	P1	P2	P3	P5	P6	P7	P11
anti-Diphtheria-toxin-Ab (IU/mL) (>0,1)	neg.	0,22	n.d.	n.d.	n.d.	0,16	n.d.
anti-Tetanustoxin-Ab (IU/mL) (>0,1)	neg.	0,29	n.d.	n.d.	n.d.	0,19	n.d.
VZV IgG (mU/mL) (negative= < 60)	neg.	132	n.d.	pos.	n.d.	neg.	n.d.
EBV (VCA) IgG (U/ml) (negative= < 9)	n.d.	neg.	pos.	n.d.	n.d.	neg.	n.d.
CMV IgG U/mL (>15)	pos.	n.d.	pos.	neg.	n.d.	n.d.	n.d.
HBS-Ab (IU/L) (>100)	neg.	150	n.d.	neg.	146,58	neg.	n.d.
Morbilli IgG (U/mL) (negative= <0,15)	neg.	169	n.d.	neg.	n.d.	n.d.	n.d.
Rubeola IgG-Ab titer (>10)	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
HiB IgG (mg/L) (≥0,15)	neg.	4,2	n.d.	n.d.	n.d.	<0,11	<0,11
Pneumococci IgG (mg/L) (negative, >15-270)	pos.	14,6	n.d.	n.d.	n.d.	n.d.	n.d.

Ab=antibodies, IU=international unit, mU=milliunit, neg.= negative, pos.= positive

P1 showed an overall poor vaccination status and tested negative for anti-Diphteriatoxoid-antibodies, anti- Tetanustoxoid-antibodies, Varizella zoster virus (VZV) IgG, Hepatitis B surface antibodies (HBs Ab), Measle (Morbilli) antibodies and Hemophilus influenzae B (HiB) IgG. No sufficient protection concerning these pathogens is therefore given. P1 tested positive for CMV IgG, Rubeola IgG and Pneumococci IgG. P2 only tested negative for EBV IgG and the evaluated Pneumococci IgG level (14,6 mg/L) was on the lower side of a normal reaction (>15-270 mg/L). The rest of the evaluated vaccination and serostatus (Diphteria, Tetanus, VZV, Hepatitis B, Measles, HiB, Pneumococci) was found to be normal in terms of antibody serum levels detected. P3 was not tested for anything but EBV IgG and CMV IgG and tested positive for both. P5 showed negative serum levels of CMV IgG, HBs Ab and Morbilli IgG. A positive result for VZV IgG was furthermore found in this patient. All other requested parameters were not tested. The only parameter available of P6 was a HBs Ab, that can be referred to as positive and normal (146,58 U/L). P7 revealed a sufficient serum level of Diphteria and Teatanus antibodies and

negative or non-sufficient levels of VZV IgG, EBV IgG, HBs Ab and HiB IgG. Non-sufficient levels of HiB IgG were furthermore detected in P11.
The available vaccination and serostatus revealed a lot of negative or non-sufficient results overall. Three out of four patients tested showed negative/non-sufficient serum levels of HiB IgG. Two out of three tested patients revealed no sufficient protection for Measles. HBs Ab were not detectable in three out of five patients tested. Half of the patients (two out of four) where serum levels of VZV IgG were available showed negative results.

4.3.3.2 Other serology parameters/ Autoimmunity

Anti nuclear antibodies (ANA) were tested in four patients. Two results (P2, P7) were negative and two patients (P6, P11) tested positive for ANA. AntidsDNA was negative in P2, P6 and P7 and positive in P11. P11 was therefore tested positive for both ANA and antidsDNA, but no clinical signs of autoimmunity were observed. Cardiolipin IgG and Cardiolipin IgM were negative in P2 and P6. P2 furthermore showed negative b2-GP-IgG and b2-GP-IgM. Information of no other patient was available concerning autoimmunity or other requested serology parameters as described in Chapter 3.

4.4 Excluded patients

Three patients, of whom data were collected, could not be included in this study because the genetic diagnosis of CMMRD was not made, but shall be presented shortly in this thesis as well. As per **Table 15**, the group of excluded patients was composed of one female and two male patients. Two patients were found to be heterozygous MMR gene mutation carriers. P2excl., the mother of P3, who was presented to as a possible CMMRD patient, carried a monoallelic *MSH6* mutation. Only one *MLH1* mutation was detected in P3excl, a 14-year-old boy who got diagnosed with Gliomatosis cerebri aged 12. P1 excl., a 9-year-old boy, who developed a brain tumour of the mesencephalon was not found to carry a mutation in one of the MMR genes, with no futher information avaible. Both known malignancies (P1excl., P3excl.) in this group were thus malignant brain tumours. The only nonmalignant feature in this group was hyperpigmentation exhibited by P1excl.. No remarkable results concerning both the clinical as well as the cellular/humoral immunophenotype could be detected in P1excl. and P3excl., where data were obtainable.

Table 15: Patient characteristic: Excluded patients (n=3)

Patient characteristic	P1excl.	P2excl.	P3excl.
Age (y)	9	n.a.	14
Gender (m/f)	m	f	m
Mutation	n.a.	*MSH6* heterozygous c.3261dupC p.Phe1088LeufsX5	*MLH1* heterozygous
Malignancies	Brain tumour mesencephalon	n.a.	Gliomatosis cerebri
Nonmalignant features	Hyperpigmentation	n.a.	none
Clinical immunophenotype	unremarkable	n.a.	unremarkable
Cellular/humoral phenotype	unremarkable	n.a.	unremarkable

5. Discussion

Whereas monoallelic MMR gene mutations lead to Lynch-syndrome, biallelic MMR gene mutations result in a condition referred to as CMMRD, a rare but devastating CPS, reported in a total of only 198 patients to date (36).

Most publications available, concerning CMMRD, are individual case reports, which implies that selection bias must at all times be kept in mind. They mainly concentrate on family history, diagnostic approach, genetics, clinical features, therapeutic approach and surveillance (26, 36, 44, 61). This case series including 11 unselected CMMRD patients from 11 families and 8 different centers across Europe and the Middle East reinforces and corroborates previous clinical findings and descriptions in CMMRD patients (36, 38, 64). But even more, it approaches to add new facets of knowledge by giving insights into immunological aspects, both clinical as well as cellular and humoral, of patients suffering from this CPS, as MMR is in fact involved in B- cell maturation, more specifically CSR and SHM. It thus represents a link between DNA repair, the immune system and cancer (34, 82).

5.1 Clinical findings

Concerning the underlying biallelic mutation, the foremost MMR gene mutated in CMMRD patients according to publications is *PMS2* (58%), in contrast to LS where most of the patients are said to carry heterozygous *MLH1* or *MSH2* mutations. The distribution of the remaining mutations in CMMRD is however estimated to be approximately balanced between *MSH2/MLH1* and *MSH6* (36, 39). With 55 % of this study population being biallelic *PMS2* mutation carriers, 27% showing a biallelic *MSH6* mutation and 18% carrying a biallelic *MLH1* mutation, the findings of this thesis are in concordance with the proposition of *PMS2* being the mutation detected the most in CMMRD. Exact reasons for that suggested discrepancy with mutations detected in LS are still not fully understood (36, 38), especially since Truninger et al. (56) revealed in 2005, that among unselected colorectal cancers monoallelic *PMS2* mutation are just as commonly found (56). Interestingly, there was no patient included carrying a homozygous *MSH2* mutation. This type of mutation is indeed observed the least and it was suggested by Bakry et al. (7) that highly penetrant, homozygous *MSH2* mutations may be letal, like some *BRCA2* mutations are when homozygous, as reported by Rahman and Scott in 2007 (60).

Two patients of our study population (P1, P8) are known to have deceased during follow-up. As a drawback of this study, survival status was not obtained of remaining patients. But as the inclusion into the study presupposed a patient alive and as there was no long follow up time this was not one of the main approaches. It is however known that survival is poor in CMMRD patients, as Lavoine et al. proposed a mean survival time of 27 months from diagnosis of first tumour to death in their cohort (64). LS related cancer history in our patient's kindred, was like other reviews previously suggested (36), mostly negative and not massively rewarding even if parents, posssibly both, of CMMRD patients carry a monoallelic mutation in a MMR gene, as proven in the family of P1 or in P3´s mother, who we excluded. Ripperger et al. proposed a negative family history in up to 85% of patients in 2010 (43) and with 9 out of 11 (81,82%) negative family histories concerning LS related cancer this high estimate was almost met, albeit in a smaller setting. Only two CRCs, in P1´s mother at 46 years and in the maternal grandfather of P6 at 40 years, both homozygous *PMS2* mutation carriers, could be detected and since only two positive family histories concerning LS related tumours, although both described in *PMS2* carriers, were detected no statement concerning an aforesaid less (36) or in this case more contributing family history in *PMS2* mutation carriers could be made. However, affected siblings, led the way to diagnosis in P3, which highlights the importance of this hint. Parental consanguinity, on the contrary, was obtained in 7 out of 9 available histories (77,78%) and our case series therefore corroborates and exceeds the high estimates of >50%, previously made (14, 41, 42, 58, 77), especially for homozygous cases and depending on the country of origin with less higher chances proposed in western countries (44), as previously shown in a French case series where only 30% were offsprings of consanguineous parents (64).

Because all 198 CMMRD patients (100%) reported to date developed at least one malignancy, the estimated cancer risk is suggested to be exceedingly high and our study population reflects this estimate as clinical presentation included malignancies in 10 out of 11 patients (90,9%) so far. Eight patients (80%) developed their first malignancy in childhood or adolescence, which underlines the fact, that CMMRD in contrast to LS is a distinct childhood cancer syndrome. P11, aged 10 and a PMS2 mutation carrier that became suspicious through CALMS and parental consanguinity only, did not develop any malignancy yet. Wimmer et al. (36) did in fact propose a tendency towards later malignancy onset in *PMS2* mutation carriers

observed to date (36). The mean age of malignancy onset in our study population was 7,4 years with a wide range of 0,66-22 years which matches the 7,5 years with an even wider range (0,4-39 years) suggested in previous studies with a larger case number(44). In concordance with Wimmer et al.´s (36) suggestion of age at malignancy onset depending on the underlying mutation, the earliest group to first develop malignancies were *MLH1* mutation carriers, followed by *MSH6* mutation carriers and with a mean age at malignancy onset of 11,6 years *PMS2* mutation carriers were the latest in this study and thus only slightly older than previously published (10 years) (36), even if reasons for this possibly later onset are not fully understood (36).

Ten patients developed a total of 20 malignancies, which included, albeit our study population was rather small, malignancies of all four groups described to occur in these patients and therefore the typical spectrum for this CPS. Seven hematological malignancies, which were diagnosed the earliest with a mean age of 4,35 years (0,66-15 yers), six malignant brain tumours with a mean age at diagnosis of 18,32 years (3-34 years), five LS associated malignancies which developed the latest with a mean age at diagnosis of 27,5 years (20-36 years) and two other tumours (Wilms tumour, malignant Phylloides tumour) were detected and the latter respectively represent only the second of this type described to date (39). As numbers of reports of CMMRD patients, including this study, are increasing, the spectrum of malignancies occurring in CMMRD is constantly growing. Even if our small study population developed malignant brain tumours and LS associated malignancies later than previously described, respectively 9,5 and 16 years, the chronology of occurrence of different types of malignancies matches previous descriptions and the wide age range we found was also reported in other cohorts (36, 38, 44, 64). In concordance with a suggested degree of genotype-phenotype correlation (36), LS associated malignancies were only found in *PMS2* mutation carriers, malignant brain tumours were mainly detected in *PMS2* mutation carriers as well and both included *MLH1* mutation carriers developed hematological malignancies. They were, however, not the only ones as this was the group of malignancies detected the most often, with T-NHL diagnosed four times being the most frequently detected hematological malignancy, like in previous descriptions (36), and second most frequently detected malignancy overall in this case series after high grade gliomas detected five times, which represents the malignancy detected in most CMMRD

patients to date according to Wimmer et al. who reviewed all 198 reported cases to date (39). Multiple malignancies, just like proposed, were also mostly found in *PMS2* mutation carriers (3 out of 5 patients), as they are said to more often survive their first malignancy. Some, to this effect, propose *PMS2* mutations to result in the less "severe" phenotype on grounds of *PMS2* being a less penetrant mutation, even if in our small study population other mutation carriers developed multiple malignancies as well and it ought to be kept in mind, that more than half of our cohort and the patients reported to date were in fact *PMS2* mutation carriers and therefore represented the majority of patients detected, which might have led to a distorted picture itself. *PMS2* mutation carriers are however said to show more of a Turcot-like phenotype, meaning that GI malignancies in combination with malignant brain tumours at an early age are more likely to raise suspicion than e.g. hematological malignancies in childhood and that they are therefore more often tested regarding a CPS on grounds of multiple malignancies and thus probably just diagnosed more often (39). Two *PMS2* mutation carriers (P1 and P6), aged above 18, exactly displayed this phenotype first picked up by Turcot (47). Treatmentwise, chemotherapy was administered in all six patients were data were obtainable. Glioblastomata in P1, which she eventually died from, and P6 were treated with radiotherapy and temozolamid, even if the usage of methylating agents, which are indeed commonly utilized in glioblastoma treatment, in mismatch repair deficient patients is highly debated. It was shown by many studies, that MMR deficient cells are more resistant towards these agents (27, 61, 79) since the antineoplastic activity of temozolamid depends, as Schiff et al. (159) state, on MMR which might make treatment ineffective. Even more, negative effects such as increased tumour mutagenesis after treatment were observed (81, 159).

CALMS, reminiscent of NF-1, were detected in 62-97% of CMMRD patients in other study cohorts (7, 36, 39, 64). This nonmalignant feature, found in 20% of schoolkids in a study conucted by Merks et al.(36, 160), was exhibited in 10 out of 11 patients (90,9%) in our case series. A possible explanation for that feature exhibited by almost all CMMRD patients and other NF-1 like features (e.g. Lisch nodules, freckling, tibial pseudoarthrosis) detected in CMMRD patients (39), although not in our study population, was given by Wang et al.(161) . They suggest that the NF1 gene is a mutational target of MMR deficiency and its inactivation leads to a NF-1 like presentation (161). Furthermore hypopigmentation, i.e. ash leaf spots and skin

pigmentation disorder, in two out of 11 patients was, amongst others, detected and this finding approximately matches the 20% proposed for a bigger cohort (64). Adenoma sebaceum and hepatic hemangioma, whereas to date only descriptions of hepatic adenomas in CMMRD patients exist (36), in P1 and Vascular malformation, and varicosis in P7 were new features detected and enlargen the spectrum of nonmalignant features observed. The only three premalignancies detected in this study population were, as most commonly found in CMMRD, different adenomas of the GI tract (dysplastic adenoma of the colon, villous adenoma in the small intestine and multiple adenomas in both the small and the large bowel) in three patients, two of which were *PMS2* mutation carriers and already developed GI malignancies. The transformation into carcinomas, however, is unfortunately found to be extremely rapid in CMMRD patients (39, 63, 67). This is probably caused by the acquisition of a somatic mutation in the polymerase proofreading genes polymerase δ and ε in the tumour in combination with the existing DNA MMR defect which in turn results in an ultrahypermutated tumour as suggested by Shlien et al. (42, 68). Thus, the remaining patient, carrying a *MLH1* mutation, will probably develop GI malignancies in the near fututure and should be under surveillance.The overall young mean age of our cohort and the short follow up time might also explain why other studies conducted in France (64) and Israel (38), in comparison to our study, detected a higher percentage of CRC. As all three patients included aged above 18 showed either GI premalignancies, GI malignancies or both, it is to expect that in patients who survive they will still develop later in their life which emphasizes the importance of surveillance. Effectiveness of suggested surveillance programms in CMMRD is not yet evidence based, but surveillance for colon tumours already showed success (42, 61, 77).

Aforesaid clinical findings strengthen previous descriptions of clinical presentation, as our cohort, even if it was composed of only 11 individuals, approximately reflects the genotype-phenotype correlation of mutations and type and number of malignancy as well as the respective age at malignancy diagnosis and other indicative features suggested in reviews including larger numbers of patients (7, 26, 36, 39, 64). Furthermore, indication criteria for testing ($\geq$ 3 points) for CMMRD proposed by the European Consortium for CMMRD in 2014 (36) and determined by phenotypical presentation and history were clearly met by all patients we presented

at the point of inclusion into this study. Mild Ig-CSR deficiency is amongst those criteria (36) and will be discussed in the following.

5.2 Immunological findings

The systematic analysis of the in vivo immunophenotype of this unselected group of patients conducted was based on both the knowledge about MMR contributing to B-cell maturation, as well as existing reports on immunological findings in 13 CMMRD patients to date with a lack of clinical descriptions and systematic analyses of the whole in vivo immunophenotype of this patient population.

Since the first immunological finding, symptomatic IgA deficiency, was described in a *MSH2* mutation carrier in 2002 by Whiteside et al. (134), research mainly concentrated on studies concerning in vitro CSR and SHM generation in MMR deficient mouse models (106, 135, 136, 143), as well as in selected MMR deficient patients, which both partially enlightened the role of MMR in CSR and SHM.

Our study aimed at analyzing B cell subsets, immunoglobuline profiles and other serology parameters, the clinical immunophenotype as well as an additional T cell immune status of CMMRD patients to obtain evidence of or to exclude a clinically relevant immunodeficiency in CMMRD.

There are solely two studies that selectively looked at B-lymphocytes, in particular circulating B- cell subsets, of CMMRD patients to date. Peron et al. (34) analyzed, in addition to conducted in vitro studies, in vivo antibody maturation in 3 PMS2 deficient patients whereas Gardes et al.(123) analyzed 8 MSH6 deficient patients.

Flowcytometric analysis of circulating B-cell subsets in immunodeficient patients has in general increasingly taken center stage of research in the past years (110, 150) and was thereofore evalued in this case series as well. It ought to be kept in mind though, that only 2% of total lymphocytes are circulating in the periphery and that the comparison of results is often somewhat limited, since used antibodies against epitopes might differ between studies conducted (109, 110). Litzman et al. states, that even the diurnal variation might lead to slightly differing results (162).

B- lymphopenia was detected in six out of 11 patients (54,55%), four of which were even considered to have a severly reduced absolute number of B- cells, whereas in the study conducted by Peron et al. (34) all three patients showed normal numbers of CD19+ B-lymphocytes. B-lymphopenia, is indeed a sign commonly found in PID such as recessive and x-linked agammaglobulinemia (163), but in Ig CSR

deficiencies this finding is in general not amongst the main characteristics (142). In CVID, which appears similar to some types of HIGM and has already been described as a possible PID phenotype in MMR deficient patients before (82, 126), the number of B-cells in peripheral blood is found to be normal or near normal in 90%. Very low B cell counts that make <1 % of lymphocytes can be caused by defects during early B-cell differentiation in the bone marrow, defective peripheral survival factors or negative regulators of these same factors (150). However, as Caver et al. (164), amongst others, showed, especially B-Lymphopenia is also a typical finding in patients who receive chemotherapy (164). Two of the patients with severe B-lymphopenia (P3, P8) are known to have received chemotherapy, but the exact time and distance to their blood test was not obtainable. Rituximab, an anti-CD 20 chimeric antibody used for immunosuppresion, has been shown to cause prolonged B-cell depletion that can, as Yoon et al. observed, last up to years (165). P3 and P8 did receive immunosuppression during chemotherapy, and although the agent was not clearly defined, this could have contributed to the present condition. Cortison, administered in P3 and P8 as well, is also known to contribute to Lymphopenia, albeit studies revealed, that T-lymphocytes are more affected (166). T-lymphocytes in this study cohort were reduced in six patients (54,55%), two of which have indeed gotten cortison (P3, P8).

As detailed information concerning the time of application of these aforesaid agents was not obtainable, a clear statement concerning the relevance of the detected cases of B-lymphopenia regarding immunodeficiency cannot be made, but of course administration itself could have been a contributing factor to the low B-cell values and T-cell values observed.

Knowing the role of MMR proteins in CSR as a backup for AID induced DNA mismatch recognition and a converter of SSB (34), the importance of looking at memory B- cells, recognizable by the surface marker CD27 (112), and especially class- switched memory B- cells appears obvious. Peron et al. (34) revealed low memory B cells in two out of three CMMRD patients, albeit most of the memory B cells were IgM+ IgD+ with low numbers of IgA+ switched B- cells observed in all patients. In the study population of Gardes et al. (123) seven out of eight patients showed normal numbers of B- memory cells whereas IgM- IgD- switched memory cells were found to be low in seven out of eight patients. They could thus both show

a clear reduction of memory B-cells that have undergone CSR successfully in 9 out of 11 (81,82%) biallelic PMS2 and MSH6 mutation carriers, although the small case number still poses a limitation in terms of interpretation.

We revealed a reduced number of ncsBm cells in four patients (P1, P2, P6, P7), albeit in all cases combinated with low csBm, since there is no such defect causing selective reduction of CD27+ IgM+IgD+ cells known to date (150). CsBm cells as measured in percentage of total B cells with values below the 5th percentile of age dependent reference values (112, 150) were observed in five patients (P1, P2, P4, P6, P7) out of 10 (50%), where subsets were detectable. This implies impaired CSR and a non-sufficient germinal center reaction in those individuals (150). A severe reduction of switched memory cells according to EUROclass (151) (<2 % of total B-cells) could however only be detected in P1 and P2 (20%). A study conducted by Huck et al. (112), in which reference values for 166 healthy children of five age groups were determined, addressed this issue in 2009. CD27+IgD+ memory cells and CD27+IgD- cells were found to show a massive increase with age and therefore Huck et al. proposed to use age adapted reference values or controls in pediatrics (112), which Peron et al. (34) and Gardes et al.(123) also did in their studies, albeit others than we used. The 2% EUROclass limit thus seems to be too high for young children, as apparent from our case series, and a cut off value for “severe reduction” in pediatric patients is however not yet determined but subject of research (112). Naive B- cell levels, that were not reported in CMMRD patients to date, are known to decrease with age (167) and were clearly increased in five patients (P1, P2, P4, P6, P7) in this case series. Interestingly, they were increased in the same patients in which we detected reduced numbers of csBm and with a nearly exact negative correlation. Litzman et al. (162) described this negative correlation in CVID patients before and it might be a compensation mechanism for a present antibody deficiency (162, 168). Circulating activated B- cells were increased in four patients (P1, P2, P4, P6), 40%, speaking for a chronic immune activation in those patients (150), which are again the same in which other relevant alterations of B-cell subsets regarding immunodeficiency were found. This group makes up approximately half of our study population. P8, who showed clinical signs of secondary probably therapy related immunodeficiency, and P9 revealed an expansion of transitional B-cells, >9% of B-cells according to EUROclass(169). This finding is detected in the

more severe cases of immunodeficiency as well. However, the underlying defects and reasons for this expansion are not fully revealed (169).

CSR deficiency, which was partially shown in five patients with reduced numbers of circulating memory cells that have undergone class switch, would logically result in defective switched isotype (IgG/IgA/IgE) production (82). Alterations in Ig levels of CMMRD patients were reported more often than in B- cell subpopulations. Decreased IgG levels, especially IgG2 and IgG4, decreased IgA or respectively IgA deficiency and particularly in young children, high IgM levels, resembling HIGM were reported in 14 patients to date and these alterations, typical for CSR deficiencies, therefore made it into the criteria for testing for CMMRD mentioned above. Since these findings are not too seldom in general population, they only account forone point (36, 39).

Reports about CMMRD patients suffering from Ig deficiencies therefore do exist, but complete and systematically analyzed immunoglobuline profiles were again only found in the two studies mentioned above (Peron et al., Gardes et al.) (34, 123).

Our results revealed mild abnormalities in the serum Ig level assay in 8 out of 10 patients (80%), where data were available. As a drawback of this study, IgG subclasses and antibodies against vaccination antigens were only available in 5 and 7 patients, respectively. Interestingly, only two of the patients (P2, P7), who showed B- cell subset alterations indicating CSR deficiency, also revealed Ig deficiencies and marked alterations in their Ig level assay. Serological data of P4 was however missing and IgG subclasses were not defined in P6. The cellular and humoral immune phenotype were therefore not found to correlate as much as expected in our case series, which strengthens the hypothesis, that CSR deficiency in CMMRD patients is only partially defective in vivo and does not markedly impair the B-cell function and Ig maturation of this patient population.

The biggest contrast however when comparing the present Ig level assay results to studies conducted before is, that we only revealed elevated IgM, resembling HIGM, in two (P1, P2) out of 10 tested patients (20%). However, neither in P1 nor P2 combinated with the suggested low IgG. Previous reports in contrast, revealed high levels of IgM in seven out of 11 tested patients (63,64%) and therefore the majority, like one would suspect in an individual with impaired CSR. It is furthermore said, that high IgM is more likely to be found in younger patients (39), but in our study population the oldest patient included (P1), aged 38 showed increased IgM levels,

but normal levels of IgG and IgA. P2, the second patient to exhibit elevated IgM levels, aged 4, was furthermore found to show absent IgA which in combination with his severly decreased csBm would represent a typical phenotype found in CSR deficiency. His normal IgG levels might thus be induced by IgG substitution, but no information concerning administration thereof was available. Additionally, P5 (10y) exhibited a phenotype resembling HIGM, with low levels of IgG and IgA and normal levels of IgM. The youngest patient (1y) included, P8, showed decreased levels of IgG, IgA, and in addition IgM, which can be found in 50% of CVID patients. In combination with the detected severe panlymphopenia, these results might though, like aforesaid, very likely be caused by administration of chemotherapy and immunosuppression. With low IgG and low IgM, a CVID-like immunoglobuline profile (112, 163) was displayed by P7 and P9. Gardes et al. and Peron et al. (34, 123), in contrast, did not observe any IgM reductions in their study populations. Since csBm cells accumulate with age, it is suggested, that Ig levels vary with age as well, like observations in MMR deficient mice demonstrated previously (34, 101, 143). IgA deficiency was indeed observed in younger patients only (1y, 4y, 10y) and IgG deficiency was apart from P7 (21y) also observed in younger patients (10y, 1y, 3y). An Ig level assay at a later point in time would be interesting in these patients, as sometimes alterations are then not detected anymore (34). IgG subclasses in our case series were again found to match and strengthen previous descriptions of immunological findings in CMMRD patients. IgG1 was normal in all patients but one (P1) and IgG3 was normal in all patients, whereas IgG2 was reduced in two out of five patients and IgG4 was reduced in four out of five patients, two of which were adults. However, it ought to be mentioned again that IgG4 levels are often not detectable in children until the age of 12-14 years, when their plateau is reached and a clinical relevance of this selective deficiency is highly debated (154).

Total IgG reduction, detected in four patients of our case series (40%), and IgA reduction or absency, observed in three patients (30%), were only detected in symptomatic patients in Peron et al.´s study (34), where those three out of 9 patients even needed Ig substitution. Even more, their clinical presentation led to the diagnosis of PID before they were diagnosed with CMMRD. All biallelic MSH6 mutation carriers studied by Gardes et al.(123) were, on the other hand, not prone to infections. This led to the assumption that PMS2 mutations would result in a clinically more severe immunophenotype, which was however never systematically

analyzed in CMMRD patients, even though several studies observed severe infections in their cohort (64) or reported on patients with known PID such as IgA deficiency (51, 124, 134).

This thus represents the first study to systematically analyze the clinical immunophenotype of an unselcted group of CMMRD patients. As a limitation of this study, only 8 out of the 11 included patients could be analyzed.

Our results surprisingly revealed no remarkable clinical immunophenotype in any of the patients, no matter which underlying mutation they carried or which laboratory alterations they revealed. All observed abberations, such as increased frequency of infections, hospitalization for infections, delayed response to antibiotics, complicated infections caused by opportunistic agents and requirement for immunosuppresion, mainly observed in P3 and P8, were most likely therapy related as they were only observed during or after treatment, respectively chemotherapy. The findings in B-cell subsets in two patients (P1, P2) of our cohort have in other PID studies been shown to markedly correlate with clinical findings. Severly low numbers of memory B-cells, especially csBm cells (<2% of B-cells), in CVID patients for instance, correlate, according to literature, with a more severe clinical phenotype, splenomegaly, lymphadenopathy, susceptibility to granulomatous diseases, lower numbers of Ig levels and lower numbers of B-lymphocytes (150, 170) and Alachkar et al. (171) could also find an increased incidence of splenomegaly and bronchiectasis in the 27 children with CVID or selective antibody deficiency that showed low levels of switched memory cells, he studied. IgA deficiency with a maximum serum level of 0,05g/l, diagnosed in P2 who presented with respiratory tract infections, albeit not recurrent and simply viral, is anyhow only symptomatic in 30% of patients(113, 125). But in our case series even patients with low IgG and IgM were not specifically prone to recurrent bacterial infections as suggested in literature (172). Proposed clinical correlations as suggested for the aforesaid PID do therefore not apply to our case series, even if laboratory parameters do partially indicate a role for MMR in antibody maturation in vivo. Still, no remarkable clinical signs are present. A possible explanation for that phenomen is, that apart from MMR the BER pathway exists to deal with AID induced mismatches (97). Furthermore, Gardes et al. (123) state, that in absence of a respective mutated MMR protein, other proteins of that pathway might also be compensating (123). The clinical immunophenotype observed in CMMRD might therefore not be very distinct. The

distinct cases Peron et al. (34) described in biallllelic PMS2 mutation carriers were, in contrast to our study, known PID patients, with a subsequently detected underlying biallelic MMR mutation.

Most infections we observed were indeed simply viral and frequency varied between 0-7 per year. An increased frequency was only specifically mentioned in P8 since chemotherapy. Four out of eight patients were described to show a “normal” frequency of infections per year by their physician in charge. It is however always challenging to distinguish between real symptoms of antibody deficiency and physiological susceptibility to infections in childhood (112). Furthermore, a distinction between side effects of treatment or malignancy and signs of immunodeficiency, that might even increase susceptibility to infections during chemotherapy in CMMRD patients (123), also makes interpretation more challenging.

Interestingly, P8, who was before chemotherapy not found to show an increased frequency of infections but now shows a susceptibility towards opportunistic agents, was the only patient to exhibit restricted TCR diversity patterns, which were previously described to be correlated with this exact clinical phenotype by Hsieh, M.Y. et al. (173). Other T-cell abberations, that are not correlated with CSR deficiency, such as T- lymphopenia, inverted or elevated CD4/CD8 ratio revealed in this study population might be secondary to an increased antigen exposure (112), administered therapy agents in patients with inverted CD4/CD8 ratio, or merely caused by underlying T-cell malignancies, like in P8 who showed an elevated CD4/CD8 ratio. A reduced number of CD4+ cells in combination with a relatively increased number of CD8+ cells was previously described in CVID and IgAD patients as well (162).

Obtained vaccination response was overall found to be poor in the seven patients evalued, which is in concordance with descriptions of PID patients in literature, who are not able to build IgG against vaccines (172). But since information about administered vaccines as well as several parameters were missing, no clear statement regarding immunodeficiency could be made in this context.

Since a predisposition towards autoimmunity or symptoms thereof is established in antibody deficiency (125), signs of autoimmunity were evalued in our case series. Two CMMRD patients who developed SLE were reported to date (36, 123). The two patients of our case series who revealed positive ANA (P6, P11), one of which also

revealed positive anti-dsDNA (P11), are however not known to have exhibited any clinical signs of autoimmunity (36, 123). But as a limiting factor, no clinical questionnaire of P6 was available.

The process of gathering data in this retrospective multicenter study, that included centers across Europe and the Middle East, itself represented one of the biggest limitations of this study. A lot of requested data and parameters were not available in retrospective or, as in some cases, never determined. The lack of these data thus made interpretation and comparison in this small and heterogenous group of patients challenging. Another major drawback of this study was, that the partial in vivo impairment of CSR we observed could not be compared with data concerning in vitro generation of CSR and SHM in this case series composed of unselected patients. Since CSR has been shown to be impaired in vitro in all patients studied to date (142), it would have been elucidating to see results of such a heterogenous group with different underlying mutations. To date studies suggest that PMS2 mutations in humans lead to CSR defects only whereas MSH6 was demonstrated to also affect SHM in humans. MSH2 deficiency was to date only studied in double knockout mice (UNG/MSH2) and affected both processes as well (34, 123, 140, 143). The different underlying MMR gene mutations did, however, not appear to have a differing effect on both the in vivo cellular/ humoral immunophenotype as well as the clinical one in our study.

This case series composed of 11 unselected CMMRD patients from 8 different centers across Europe and the Middle East corroborates previous clinical descriptions of this patient population. Furthermore, it enlarges the spectrum of reported patients, which in a rare disease like this makes a valuable contribution, not only to raise awareness, but to increase knowledge of the natural history of this condition.

Analysis of immunological aspects, to conclude, revealed no uniform cellular/humoral immunophenotype in this case series. In particular, no evidence of a potentially expected Hyper-IgM-like pattern could be detected. Partial in vivo CSR impairment is however, in concordance with previous studies (34, 123), implied by the present results including reduced circulating memory cells that have undergone class switch and Ig deficiencies. Lacking data of in vitro studies of CSR and SHM generation in these individuals represent a limitation, when interpretating the role

MMR plays in antibody maturation. Clinical correlates regarding immunodeficiencies, that were systematically analyzed in CMMRD patients for the first time in this study, were uniformly found unremarkable. Further, preferably prospective, studies with larger patient numbers would be needed in order to add evidence to the knowledge, both clinical as well as immunological, of this rare but devastating syndrome, that is to date markedly based on observations.

6. References

1. Saletta F, Dalla Pozza L, Byrne JA. Genetic causes of cancer predisposition in children and adolescents. Transl Pediatr. 2015;4(2):67-75.
2. Frank SA. Genetic predisposition to cancer - insights from population genetics. Nat Rev Genet. 2004;5(10):764-72.
3. Anderson DE. The role of genetics in human cancer. CA Cancer J Clin. 1974;24(3):130-6.
4. Hodgson S. Mechanisms of inherited cancer susceptibility. J Zhejiang Univ Sci B. 2008;9(1):1-4.
5. Dixit A, Yi L, Gowthaman R, Torkamani A, Schork NJ, Verkhivker GM. Sequence and structure signatures of cancer mutation hotspots in protein kinases. PLoS One. 2009;4(10):e7485.
6. Rahman N. Realizing the promise of cancer predisposition genes. Nature. 2014;505(7483):302-8.
7. Bakry D, Aronson M, Durno C, Rimawi H, Farah R, Alharbi QK, et al. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. European journal of cancer. 2014;50(5):987-96.
8. Broca P. Traits, des tumeurs1866.
9. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68(4):820-3.
10. Rao A, Rothman J, Nichols KE. Genetic testing and tumor surveillance for children with cancer predisposition syndromes. Curr Opin Pediatr. 2008;20(1):1-7.
11. Malkin D, Nichols KE, Zelley K, Schiffman JD. Predisposition to pediatric and hematologic cancers: a moving target. Am Soc Clin Oncol Educ Book. 2014:e44-55.
12. Skokowa J, Steinemann D, Katsman-Kuipers JE, Zeidler C, Klimenkova O, Klimiankou M, et al. Cooperativity of RUNX1 and CSF3R mutations in severe congenital neutropenia: a unique pathway in myeloid leukemogenesis. Blood. 2014;123(14):2229-37.
13. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809-21.
14. Wimmer K, Kratz CP. Constitutional mismatch repair-deficiency syndrome. Haematologica. 2010;95(5):699-701.
15. Seif AE. Pediatric leukemia predisposition syndromes: clues to understanding leukemogenesis. Cancer Genet. 2011;204(5):227-44.
16. Villani A, Shore A, Wasserman JD, Stephens D, Kim RH, Druker H, et al. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. Lancet Oncol. 2016;17(9):1295-305.
17. Stieglitz E, Loh ML. Genetic predispositions to childhood leukemia. Ther Adv Hematol. 2013;4(4):270-90.
18. Slatter MA, Gennery AR. Primary immunodeficiencies associated with DNA-repair disorders. Expert Rev Mol Med. 2010;12:e9.

19. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature. 1998;396(6712):643-9.
20. Kennedy RD, D'Andrea AD. DNA repair pathways in clinical practice: lessons from pediatric cancer susceptibility syndromes. J Clin Oncol. 2006;24(23):3799-808.
21. Jiricny J. Postreplicative mismatch repair. Cold Spring Harb Perspect Biol. 2013;5(4):a012633.
22. Watson JD, Crick FH. The structure of DNA. Cold Spring Harb Symp Quant Biol. 1953;18:123-31.
23. Pray LA. Discovery of DNA structure and function: Watson and Crick. Nature Education. 2008;1(1):100.
24. Kunkel TA, Erie DA. DNA mismatch repair. Annu Rev Biochem. 2005;74:681-710.
25. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol. 2003;21(6):1174-9.
26. Wimmer K, Etzler J. Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? Hum Genet. 2008;124(2):105-22.
27. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006;7(5):335-46.
28. de la Chapelle A. Microsatellite instability. N Engl J Med. 2003;349(3):209-10.
29. Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonucleolytic function of MutLalpha in human mismatch repair. Cell. 2006;126(2):297-308.
30. Jiricny J. MutLalpha: at the cutting edge of mismatch repair. Cell. 2006;126(2):239-41.
31. Surtees JA, Argueso JL, Alani E. Mismatch repair proteins: key regulators of genetic recombination. Cytogenet Genome Res. 2004;107(3-4):146-59.
32. Stojic L, Brun R, Jiricny J. Mismatch repair and DNA damage signalling. DNA Repair (Amst). 2004;3(8-9):1091-101.
33. Zlatanou A, Despras E, Braz-Petta T, Boubakour-Azzouz I, Pouvelle C, Stewart GS, et al. The hMsh2-hMsh6 complex acts in concert with monoubiquitinated PCNA and Pol eta in response to oxidative DNA damage in human cells. Mol Cell. 2011;43(4):649-62.
34. Peron S, Metin A, Gardes P, Alyanakian MA, Sheridan E, Kratz CP, et al. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. J Exp Med. 2008;205(11):2465-72.
35. Bodo S, Colas C, Buhard O, Collura A, Tinat J, Lavoine N, et al. Diagnosis of Constitutional Mismatch Repair-Deficiency Syndrome Based on Microsatellite Instability and Lymphocyte Tolerance to Methylating Agents. Gastroenterology. 2015;149(4):1017-29 e3.
36. Wimmer K, Kratz CP, Vasen HF, Caron O, Colas C, Entz-Werle N, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). Journal of medical genetics. 2014;51(6):355-65.
37. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clinical genetics. 2009;76(1):1-18.

38. Baris HN, Barnes-Kedar I, Toledano H, Halpern M, Hershkovitz D, Lossos A, et al. Constitutional Mismatch Repair Deficiency in Israel: High Proportion of Founder Mutations in MMR Genes and Consanguinity. Pediatric blood & cancer. 2016;63(3):418-27.
39. Wimmer K, Rosenbaum T, Messiaen L. Connections between constitutional mismatch repair deficiency syndrome and neurofibromatosis type 1. Clinical genetics. 2016.
40. Urganci N, Genc DB, Kose G, Onal Z, Vidin OO. Colorectal Cancer due to Constitutional Mismatch Repair Deficiency Mimicking Neurofibromatosis I. Pediatrics. 2015;136(4):e1047-50.
41. Durno CA, Sherman PM, Aronson M, Malkin D, Hawkins C, Bakry D, et al. Phenotypic and genotypic characterisation of biallelic mismatch repair deficiency (BMMR-D) syndrome. European journal of cancer. 2015;51(8):977-83.
42. Durno C, Boland CR, Cohen S, Dominitz JA, Giardiello FM, Johnson DA, et al. Recommendations on Surveillance and Management of Biallelic Mismatch Repair Deficiency (BMMRD) Syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer. Gastroenterology. 2017.
43. Ripperger T, Beger C, Rahner N, Sykora KW, Bockmeyer CL, Lehmann U, et al. Constitutional mismatch repair deficiency and childhood leukemia/lymphoma--report on a novel biallelic MSH6 mutation. Haematologica. 2010;95(5):841-4.
44. Tabori U, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T, et al. Clinical Management and Tumor Surveillance Recommendations of Inherited Mismatch Repair Deficiency in Childhood. Clin Cancer Res. 2017;23(11):e32-e7.
45. Ricciardone MD, Ozcelik T, Cevher B, Ozdag H, Tuncer M, Gurgey A, et al. Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. Cancer Res. 1999;59(2):290-3.
46. Wang Q, Lasset C, Desseigne F, Frappaz D, Bergeron C, Navarro C, et al. Neurofibromatosis and early onset of cancers in hMLH1-deficient children. Cancer Res. 1999;59(2):294-7.
47. Turcot J, Despres JP, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. Dis Colon Rectum. 1959;2:465-8.
48. Rana HQ, Syngal S. Biallelic Mismatch Repair Deficiency Syndrome- Management and Prevention of a Devastating Manifestation of Lynch syndrome. Gastroenterology. 2017.
49. Kruger S, Kinzel M, Walldorf C, Gottschling S, Bier A, Tinschert S, et al. Homozygous PMS2 germline mutations in two families with early-onset haematological malignancy, brain tumours, HNPCC-associated tumours, and signs of neurofibromatosis type 1. European journal of human genetics : EJHG. 2008;16(1):62-72.
50. Bandipalliam P. Syndrome of early onset colon cancers, hematologic malignancies & features of neurofibromatosis in HNPCC families with homozygous mismatch repair gene mutations. Familial cancer. 2005;4(4):323-33.
51. Scott RH, Mansour S, Pritchard-Jones K, Kumar D, MacSweeney F, Rahman N. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic MSH6 mutations. Nat Clin Pract Oncol. 2007;4(2):130-4.

52. Magnusson S, Borg A, Kristoffersson U, Nilbert M, Wiebe T, Olsson H. Higher occurrence of childhood cancer in families with germline mutations in BRCA2, MMR and CDKN2A genes. Familial cancer. 2008;7(4):331-7.
53. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2017;26(3):404-12.
54. Plaschke J, Engel C, Kruger S, Holinski-Feder E, Pagenstecher C, Mangold E, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. J Clin Oncol. 2004;22(22):4486-94.
55. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology. 2008;135(2):419-28.
56. Truninger K, Menigatti M, Luz J, Russell A, Haider R, Gebbers JO, et al. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology. 2005;128(5):1160-71.
57. ten Broeke SW, Brohet RM, Tops CM, van der Klift HM, Velthuizen ME, Bernstein I, et al. Lynch syndrome caused by germline PMS2 mutations: delineating the cancer risk. J Clin Oncol. 2015;33(4):319-25.
58. Durno CA, Holter S, Sherman PM, Gallinger S. The gastrointestinal phenotype of germline biallelic mismatch repair gene mutations. Am J Gastroenterol. 2010;105(11):2449-56.
59. Borras E, Pineda M, Cadinanos J, Del Valle J, Brieger A, Hinrichsen I, et al. Refining the role of PMS2 in Lynch syndrome: germline mutational analysis improved by comprehensive assessment of variants. Journal of medical genetics. 2013;50(8):552-63.
60. Rahman N, Scott RH. Cancer genes associated with phenotypes in monoallelic and biallelic mutation carriers: new lessons from old players. Hum Mol Genet. 2007;16 Spec No 1:R60-6.
61. Vasen HF, Ghorbanoghli Z, Bourdeaut F, Cabaret O, Caron O, Duval A, et al. Guidelines for surveillance of individuals with constitutional mismatch repair-deficiency proposed by the European Consortium "Care for CMMR-D" (C4CMMR-D). Journal of medical genetics. 2014;51(5):283-93.
62. Johannesma PC, van der Klift HM, van Grieken NC, Troost D, Te Riele H, Jacobs MA, et al. Childhood brain tumours due to germline bi-allelic mismatch repair gene mutations. Clinical genetics. 2011;80(3):243-55.
63. Aronson M, Gallinger S, Cohen Z, Cohen S, Dvir R, Elhasid R, et al. Gastrointestinal Findings in the Largest Series of Patients With Hereditary Biallelic Mismatch Repair Deficiency Syndrome: Report from the International Consortium. Am J Gastroenterol. 2016;111(2):275-84.
64. Lavoine N, Colas C, Muleris M, Bodo S, Duval A, Entz-Werle N, et al. Constitutional mismatch repair deficiency syndrome: clinical description in a French cohort. Journal of medical genetics. 2015;52(11):770-8.

65. Rengifo-Cam W, Jasperson K, Garrido-Laguna I, Colman H, Scaife C, Samowitz W, et al. A 30-Year-Old Man with Three Primary Malignancies: A Case of Constitutional Mismatch Repair Deficiency. ACG Case Rep J. 2017;4:e34.
66. Stoffel E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology. 2009;137(5):1621-7.
67. Levi Z, Kariv R, Barnes-Kedar I, Goldberg Y, Half E, Morgentern S, et al. The gastrointestinal manifestation of constitutional mismatch repair deficiency syndrome: from a single adenoma to polyposis-like phenotype and early onset cancer. Clinical genetics. 2015;88(5):474-8.
68. Shlien A, Campbell BB, de Borja R, Alexandrov LB, Merico D, Wedge D, et al. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. Nat Genet. 2015;47(3):257-62.
69. Baas AF, Gabbett M, Rimac M, Kansikas M, Raphael M, Nievelstein RA, et al. Agenesis of the corpus callosum and gray matter heterotopia in three patients with constitutional mismatch repair deficiency syndrome. European journal of human genetics : EJHG. 2013;21(1):55-61.
70. Alotaibi H, Ricciardone MD, Ozturk M. Homozygosity at variant MLH1 can lead to secondary mutation in NF1, neurofibromatosis type I and early onset leukemia. Mutat Res. 2008;637(1-2):209-14.
71. Wimmer K, Beilken A, Nustede R, Ripperger T, Lamottke B, Ure B, et al. A novel germline POLE mutation causes an early onset cancer prone syndrome mimicking constitutional mismatch repair deficiency. Familial cancer. 2017;16(1):67-71.
72. Sjursen W, Bjornevoll I, Engebretsen LF, Fjelland K, Halvorsen T, Myrvold HE. A homozygote splice site PMS2 mutation as cause of Turcot syndrome gives rise to two different abnormal transcripts. Familial cancer. 2009;8(3):179-86.
73. Hendriks YM, de Jong AE, Morreau H, Tops CM, Vasen HF, Wijnen JT, et al. Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. CA Cancer J Clin. 2006;56(4):213-25.
74. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature. 1993;363(6429):558-61.
75. Leenen CH, Geurts-Giele WR, Dubbink HJ, Reddingius R, van den Ouweland AM, Tops CM, et al. Pitfalls in molecular analysis for mismatch repair deficiency in a family with biallelic pms2 germline mutations. Clinical genetics. 2011;80(6):558-65.
76. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology. 2000;118(5):829-34.
77. Durno CA, Aronson M, Tabori U, Malkin D, Gallinger S, Chan HS. Oncologic surveillance for subjects with biallelic mismatch repair gene mutations: 10 year follow-up of a kindred. Pediatric blood & cancer. 2012;59(4):652-6.

78. Vasen HF, Moslein G, Alonso A, Bernstein I, Bertario L, Blanco I, et al. Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). Journal of medical genetics. 2007;44(6):353-62.
79. Fedier A, Fink D. Mutations in DNA mismatch repair genes: implications for DNA damage signaling and drug sensitivity (review). Int J Oncol. 2004;24(4):1039-47.
80. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med. 2003;348(10):919-32.
81. Hunter C, Smith R, Cahill DP, Stephens P, Stevens C, Teague J, et al. A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. Cancer Res. 2006;66(8):3987-91.
82. de Miranda NF, Bjorkman A, Pan-Hammarstrom Q. DNA repair: the link between primary immunodeficiency and cancer. Ann N Y Acad Sci. 2011;1246:50-63.
83. Howard JC. Immunology. Disease and evolution. Nature. 1991;352(6336):565-7.
84. Bonilla FA, Oettgen HC. Adaptive immunity. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S33-40.
85. Murphy K, Travers P, Walport M. Janeway´s Immunobiology. 7th ed. New York,NY: Garland Science; 2008.
86. Revy P, Buck D, le Deist F, de Villartay JP. The repair of DNA damages/modifications during the maturation of the immune system: lessons from human primary immunodeficiency disorders and animal models. Adv Immunol. 2005;87:237-95.
87. Ramsden DA, Weed BD, Reddy YV. V(D)J recombination: Born to be wild. Semin Cancer Biol. 2010;20(4):254-60.
88. Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, et al. The biochemistry of somatic hypermutation. Annu Rev Immunol. 2008;26:481-511.
89. Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A. Expression of two T cell receptor alpha chains: dual receptor T cells. Science. 1993;262(5132):422-4.
90. Melenhorst JJ, Lay MD, Price DA, Adams SD, Zeilah J, Sosa E, et al. Contribution of TCR-beta locus and HLA to the shape of the mature human Vbeta repertoire. J Immunol. 2008;180(10):6484-9.
91. McLean-Tooke A, Barge D, Spickett GP, Gennery AR. T cell receptor Vbeta repertoire of T lymphocytes and T regulatory cells by flow cytometric analysis in healthy children. Clin Exp Immunol. 2008;151(1):190-8.
92. Lefranc MP, Rabbitts TH. Two tandemly organized human genes encoding the T-cell gamma constant-region sequences show multiple rearrangement in different T-cell types. Nature. 1985;316(6027):464-6.
93. Roth DB. V(D)J Recombination: Mechanism, Errors, and Fidelity. Microbiol Spectr. 2014;2(6).
94. Puebla-Osorio N, Zhu C. DNA damage and repair during lymphoid development: antigen receptor diversity, genomic integrity and lymphomagenesis. Immunol Res. 2008;41(2):103-22.
95. Sadofsky MJ. The RAG proteins in V(D)J recombination: more than just a nuclease. Nucleic Acids Res. 2001;29(7):1399-409.

96. Durandy A, Taubenheim N, Peron S, Fischer A. Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. Adv Immunol. 2007;94:275-306.
97. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol. 2008;26:261-92.
98. Stavnezer J, Bjorkman A, Du L, Cagigi A, Pan-Hammarstrom Q. Mapping of switch recombination junctions, a tool for studying DNA repair pathways during immunoglobulin class switching. Adv Immunol. 2010;108:45-109.
99. Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. Toxicology. 2003;193(1-2):3-34.
100. Schrader CE, Guikema JE, Linehan EK, Selsing E, Stavnezer J. Activation-induced cytidine deaminase-dependent DNA breaks in class switch recombination occur during G1 phase of the cell cycle and depend upon mismatch repair. J Immunol. 2007;179(9):6064-71.
101. Rada C, Di Noia JM, Neuberger MS. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. Mol Cell. 2004;16(2):163-71.
102. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 2000;102(5):553-63.
103. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 2000;102(5):565-75.
104. Arakawa H, Hauschild J, Buerstedde JM. Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion. Science. 2002;295(5558):1301-6.
105. Fugmann SD, Schatz DG. Immunology. One AID to unite them all. Science. 2002;295(5558):1244-5.
106. Kim N, Bozek G, Lo JC, Storb U. Different mismatch repair deficiencies all have the same effects on somatic hypermutation: intact primary mechanism accompanied by secondary modifications. J Exp Med. 1999;190(1):21-30.
107. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008;112(5):1570-80.
108. Chan JK, Ng CS, Hui PK. A simple guide to the terminology and application of leucocyte monoclonal antibodies. Histopathology. 1988;12(5):461-80.
109. Gradwohl G. Hochsensitive FACS-Analyse der B-Zell Subpopulationen bei gesunden Probanden. Graz: Medizinische Universität Graz; 2004.
110. Sack U. Zelluläre Diagnostik Grundlagen, Methoden und klinische Anwendungen der Durchflusszytometrie. Basel: Karger; 2007.
111. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med. 1998;188(9):1679-89.

112. Huck K, Feyen O, Ghosh S, Beltz K, Bellert S, Niehues T. Memory B-cells in healthy and antibody-deficient children. Clin Immunol. 2009;131(1):50-9.
113. Pan-Hammarstrom Q, Hammarstrom L. Antibody deficiency diseases. Eur J Immunol. 2008;38(2):327-33.
114. Salavoura K, Kolialexi A, Tsangaris G, Mavrou A. Development of cancer in patients with primary immunodeficiencies. Anticancer Res. 2008;28(2B):1263-9.
115. Waltenburg R, Kobrynski L, Reyes M, Bowen S, Khoury MJ. Primary immunodeficiency diseases: practice among primary care providers and awareness among the general public, United States, 2008. Genet Med. 2010;12(12):792-800.
116. Vajdic CM, Mao L, van Leeuwen MT, Kirkpatrick P, Grulich AE, Riminton S. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood. 2010;116(8):1228-34.
117. Jankovic M, Nussenzweig A, Nussenzweig MC. Antigen receptor diversification and chromosome translocations. Nat Immunol. 2007;8(8):801-8.
118. Edry E, Melamed D. Class switch recombination: a friend and a foe. Clin Immunol. 2007;123(3):244-51.
119. Niehues T, Perez-Becker R, Schuetz C. More than just SCID--the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. Clin Immunol. 2010;135(2):183-92.
120. Taylor AM, Metcalfe JA, Thick J, Mak YF. Leukemia and lymphoma in ataxia telangiectasia. Blood. 1996;87(2):423-38.
121. Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell. 2001;105(2):177-86.
122. Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, et al. Omenn syndrome due to ARTEMIS mutations. Blood. 2005;105(11):4179-86.
123. Gardes P, Forveille M, Alyanakian MA, Aucouturier P, Ilencikova D, Leroux D, et al. Human MSH6 deficiency is associated with impaired antibody maturation. J Immunol. 2012;188(4):2023-9.
124. Ostergaard JR, Sunde L, Okkels H. Neurofibromatosis von Recklinghausen type I phenotype and early onset of cancers in siblings compound heterozygous for mutations in MSH6. Am J Med Genet A. 2005;139A(2):96-105; discussion 96.
125. Hammarstrom L, Smith CIE. Genetic approach to common variable immunodeficiency and IgA deficiency. In: Ochs HD, Smith CIE, Puck JM, editors. Primary immunodeficiency diseases. Oxford: Oxford University Press; 2007. p. 1207-16.
126. Offer SM, Pan-Hammarstrom Q, Hammarstrom L, Harris RS. Unique DNA repair gene variations and potential associations with the primary antibody deficiency syndromes IgAD and CVID. PLoS One. 2010;5(8):e12260.
127. Goshen E, Livne A, Krupp M, Hammarstrom L, Dighiero G, Slor H, et al. Antinuclear and related autoantibodies in sera of healthy subjects with IgA deficiency. J Autoimmun. 1989;2(1):51-60.

128. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92(1):34-48.
129. Eades-Perner AM, Gathmann B, Knerr V, Guzman D, Veit D, Kindle G, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2004-06. Clin Exp Immunol. 2007;147(2):306-12.
130. Eibl MM, Wolf HM. Vaccination in patients with primary immune deficiency, secondary immune deficiency and autoimmunity with immune regulatory abnormalities. Immunotherapy. 2015;7(12):1273-92.
131. (ESID) ESfI. ESID Registry - Working definitions for clinical diagnosis of PID 2017 [Available from: https://esid.org/Working-Parties/Registry/Diagnosis-criteria.
132. Schulze IF, S. . Infektanfälligkeit oder primärer Immundefekt? Pädiatr hautnah. 2012;24(5):320-6.
133. Farmand S BU, von Bernuth H, Borte M, Foerster-Waldl E, Franke K, Habermehl P, Kapaun P, Klock G, Liese J et al. Leitlinie zur Diagnostik von primären Immundefekten. Register-Nr 027/050. http://www.awmf.org/. Stand 01.12.2011. Leitlinie zur Diagnostik von primären Immundefekten. 2011 [Available from: http://www.awmf.org/.
134. Whiteside D, McLeod R, Graham G, Steckley JL, Booth K, Somerville MJ, et al. A homozygous germ-line mutation in the human MSH2 gene predisposes to hematological malignancy and multiple cafe-au-lait spots. Cancer Res. 2002;62(2):359-62.
135. Schrader CE, Edelmann W, Kucherlapati R, Stavnezer J. Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes. J Exp Med. 1999;190(3):323-30.
136. Li Z, Scherer SJ, Ronai D, Iglesias-Ussel MD, Peled JU, Bardwell PD, et al. Examination of Msh6- and Msh3-deficient mice in class switching reveals overlapping and distinct roles of MutS homologues in antibody diversification. J Exp Med. 2004;200(1):47-59.
137. Martomo SA, Yang WW, Gearhart PJ. A role for Msh6 but not Msh3 in somatic hypermutation and class switch recombination. J Exp Med. 2004;200(1):61-8.
138. Sekine H, Ferreira RC, Pan-Hammarstrom Q, Graham RR, Ziemba B, de Vries SS, et al. Role for Msh5 in the regulation of Ig class switch recombination. Proc Natl Acad Sci U S A. 2007;104(17):7193-8.
139. Wu X, Tsai CY, Patam MB, Zan H, Chen JP, Lipkin SM, et al. A role for the MutL mismatch repair Mlh3 protein in immunoglobulin class switch DNA recombination and somatic hypermutation. J Immunol. 2006;176(9):5426-37.
140. Ehrenstein MR, Rada C, Jones AM, Milstein C, Neuberger MS. Switch junction sequences in PMS2-deficient mice reveal a microhomology-mediated mechanism of Ig class switch recombination. Proc Natl Acad Sci U S A. 2001;98(25):14553-8.
141. Schrader CE, Vardo J, Stavnezer J. Role for mismatch repair proteins Msh2, Mlh1, and Pms2 in immunoglobulin class switching shown by sequence analysis of recombination junctions. J Exp Med. 2002;195(3):367-73.
142. Durandy A, Kracker S. Immunoglobulin class-switch recombination deficiencies. Arthritis Res Ther. 2012;14(4):218.

143. Shen HM, Tanaka A, Bozek G, Nicolae D, Storb U. Somatic hypermutation and class switch recombination in Msh6(-/-)Ung(-/-) double-knockout mice. J Immunol. 2006;177(8):5386-92.
144. Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol. 2003;4(10):1023-8.
145. Karaca NE, Durandy A, Gulez N, Aksu G, Kutukculer N. Study of patients with Hyper-IgM type IV phenotype who recovered spontaneously during late childhood and review of the literature. Eur J Pediatr. 2011;170(8):1039-47.
146. Fogarty PF, Rick ME, Zeng W, Risitano AM, Dunbar CE, Bussel JB. T cell receptor VB repertoire diversity in patients with immune thrombocytopenia following splenectomy. Clin Exp Immunol. 2003;133(3):461-6.
147. Currier JR, Deulofeut H, Barron KS, Kehn PJ, Robinson MA. Mitogens, superantigens, and nominal antigens elicit distinctive patterns of TCRB CDR3 diversity. Hum Immunol. 1996;48(1-2):39-51.
148. Ilencikova D. [Constitutional mismatch repair-deficiency syndrome (CMMR-D) - a case report of a family with biallelic MSH6 mutation]. Klin Onkol. 2012;25 Suppl:S34-8.
149. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-8.
150. Warnatz K, Schlesier M. Flowcytometric phenotyping of common variable immunodeficiency. Cytometry B Clin Cytom. 2008;74(5):261-71.
151. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111(1):77-85.
152. Stoop JW, Zegers BJ, Sander PC, Ballieux RE. Serum immunoglobulin levels in healthy children and adults. Clin Exp Immunol. 1969;4(1):101-12.
153. Safari M, Rezaei N, Hajilooi M, Aghamohammadi A, Pan-Hammarstrom Q, Hammarstrom L. Onychomadesis in a patient with immunoglobulin class switch recombination deficiency. Iran J Allergy Asthma Immunol. 2008;7(1):41-4.
154. Hayashibara H, Tanimoto K, Nagata I, Harada Y, Shiraki K. Normal levels of IgG subclass in childhood determined by a sensitive ELISA. Acta Paediatr Jpn. 1993;35(2):113-7.
155. Schur PH, Rosen F, Norman ME. Immunoglobulin subclasses in normal children. Pediatr Res. 1979;13(3):181-3.
156. Oxelius VA. IgG subclass levels in infancy and childhood. Acta Paediatr Scand. 1979;68(1):23-7.
157. Morell A, Skvaril F, Hitzig WH, Barandun S. IgG subclasses: development of the serum concentrations in "normal" infants and children. J Pediatr. 1972;80(6):960-4.
158. Siegrist CA. [Vaccinology: which catch-up plans for patients with unknown or incomplete immunizations?]. Rev Med Suisse. 2009;5(185):63-8.
159. Schiff D, Lam EM, Parisi JE, Giannini C, Uhm JH, Keegan BM, et al. A 21-year-old woman with multiple cancers and a new brain lesion. Neurology. 2012;78(10):743-9.

160. Merks JH, Ozgen HM, Cluitmans TL, van der Burg-van Rijn JM, Cobben JM, van Leeuwen FE, et al. Normal values for morphological abnormalities in school children. Am J Med Genet A. 2006;140(19):2091-109.
161. Wang Q, Montmain G, Ruano E, Upadhyaya M, Dudley S, Liskay RM, et al. Neurofibromatosis type 1 gene as a mutational target in a mismatch repair-deficient cell type. Hum Genet. 2003;112(2):117-23.
162. Litzman J, Vlkova M, Pikulova Z, Stikarovska D, Lokaj J. T and B lymphocyte subpopulations and activation/differentiation markers in patients with selective IgA deficiency. Clin Exp Immunol. 2007;147(2):249-54.
163. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol. 1999;93(3):190-7.
164. Caver TE, Slobod KS, Flynn PM, Behm FG, Hudson MM, Turner EV, et al. Profound abnormality of the B/T lymphocyte ratio during chemotherapy for pediatric acute lymphoblastic leukemia. Leukemia. 1998;12(4):619-22.
165. Yoon JYea. B Cell Lymphopenia As a Complication of Remote Rituximab Use in a Patient with Common Variable Immunodeficiency. Journal of Allergy and Clinical Immunology. 2015;135(2).
166. Gedalia A, Shetty AK. Chronic steroid and immunosuppressant therapy in children. Pediatr Rev. 2004;25(12):425-34.
167. Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol. 2010;162(2):271-9.
168. Hagspiel V. B-Zellsubpopulationen bei Patienten mit Immundefizienz. Graz: Medizinische Universität Graz; 2004.
169. Vlkova M, Thon V, Sarfyova M, Blaha L, Svobodnik A, Lokaj J, et al. Age dependency and mutual relations in T and B lymphocyte abnormalities in common variable immunodeficiency patients. Clin Exp Immunol. 2006;143(2):373-9.
170. Jacquot S, Macon-Lemaitre L, Paris E, Kobata T, Tanaka Y, Morimoto C, et al. B cell co-receptors regulating T cell-dependent antibody production in common variable immunodeficiency: CD27 pathway defects identify subsets of severely immuno-compromised patients. Int Immunol. 2001;13(7):871-6.
171. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B cell percentage and not serum immunoglobulin concentration is associated with clinical complications in children and adults with specific antibody deficiency and common variable immunodeficiency. Clin Immunol. 2006;120(3):310-8.
172. Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol. 2009;27:199-227.
173. Hsieh MY, Hong WH, Lin JJ, Lee WI, Lin KL, Wang HS, et al. T-cell receptor excision circles and repertoire diversity in children with profound T-cell immunodeficiency. J Microbiol Immunol Infect. 2013;46(5):374-81.

7. Appendix

Your Name | Institution | Contact:
Patient ID (no name) | Age:

C4CMMRD | Immunodeficiency Questionnaire & Checklist Version 1.3; Jan 23, 2017

1. **Laboratory:**
   - ○ 1.1. please analyze the cellular immune status (T, B, NK, naïve T, T and B memory cells) and send us a copy of your results; alternatively send 10-12mL EDTA peripheral blood overnight to Graz (arrival Mo-Wed 9 a.m.!, please preannounce) for these analyses – Thanks!
   - ○ 1.2. please analyze IgG, IgA, IgM, IgE, IgG-subclasses, IgG against tetanus toxoid, diphtheria toxoid, pneumococci, haemophilus infl.B, if possible also: varicella, EBV, measles, hepatitis B, isohemagglutinins, paraproteins; ANA + ENA if pos., dsDNA-Ab, Cardiolipin; compare the results to vaccination history, blood group, and send copy of results – Thanks!
2. **History** (please check the circles and fill in the data):
   - ○ 2.1. Family history of immunodeficiency or increased risk of infections
   - ○ 2.2. Hospitalizations due to infections? / for i.v. antibiotics (how often, length, ICU?)
   - 2.3. Reactions/complications after live vaccines? ○ no ○ pathologic: ___
   - 2.4. Healing of the navel/omphalos ○ normal ○ pathological (>3wks): ___
   - 2.5. Growth during childhood, percentile deviation ○ normal ○ deviant: ___
   - ○ 2.6. Infections
     - 2.6.1. Frequency ○ no ○ yes n= ___ per month, ___ per year
       first presentation of symptoms: ___
     - 2.6.2. Severity ○ simple viral ○ complicated viral ○ simple bacterial
       ○ invasive bacterial ○ invasive fungal ○ other
       describe: ___
     - 2.6.3. Localization ○ ENT ○ lungs ○ bones ○ meningitis
       ○ organ abscesses ○ other: ___
     - 2.6.4. Infectious agents
       ○ not tested ○ normal spectrum ○ opportunistic ○ name: ___
       ○ other: _____
     - 2.6.5. Response to oral or i.v. antibiotic treatment ○ normal ○ delayed ○ refractory
     - 2.6.6. Requirement of corticosteroids or immunosuppression: ○ no yes: ___
   - ○ 2.7. Autoimmunity / Immune dysregulation
     - 2.7.1. Granuloma (localization, course, histo.) ○ no ○ yes: ___
     - 2.7.2. Autoimmunity (organ, course, treatment) ○ no ○ yes: ___ describe: ___
     - 2.7.3. Relapsing fever: ○ no ○ yes: ___ (+symptoms?)
       age at initial presentation: ___ interval: ___ duration of flare: ___
     - 2.7.4. Lymphoproliferation, Splenomegaly ○ no ○ yes: ___
     - 2.7.5. Hepatopathy, Cholangitis, Cryptosporidium infection ○ no ○ yes: ___
     - 2.7.6. Inflammatory bowel disease ○ no ○ yes: ___
   - 2.8. Other (please describe and / or send medical report)
   - ○ 3. Malignoma (diagnosis and first presentation):
     ○ chemotherapy ○ radiation therapy ○ allogeneic stem cell transplantation
   - ○ 4. **Clinical / physical Status** (please describe abnormalities or attach report)
   - ○ 5. **Lung function** tests, **Chest-CT**, **Abdomen-MRI** (please attach most recent reports)

© & contact: markus.seidel@medunigraz.at | mob 0043 316 385 80215 | secr 0043 316 385 13485 | fax 0043 316 385 13717 | Pediatric Hematology-Oncology | Medical University Graz | Auenbruggerpl. 38, 8036 GRAZ, Austria

Table 16: Detailed characteristics of included patients with CMMRD (n=11)

Patient	Age	Mutation	Family tumours	Parental consan-guinity	Nonmalignant Features	Premalignancies	Malignancies	Immunological alterations regarding Ig CSR deficiency
P1	38	PMS2 c.137G>T p.Ser46Ile	LS family Mother: CRC (46y)	✓	Adenoma sebaceum Hepatic hemangioma	Dysplastic Adenomata (Colon)	CRC (22 y) Glioblastoma (34y) Duodenal Ca (36 y) Endometrial Ca (36y)	severe csBm reduction, elevated IgM, IgG1 , IgG4 deficiency
P2	4	MSH6 c.[467C>G]+[1316A>G] p.[Ser156Ter]+[Asp439Gly]	no	n.a.	Hemangioma CALMS Ash-leaf spots		Anaplastic Medulloblastoma	severe csBm reduction, IgA deficiency, elevated IgM
P3	6	MSH6 c.[3261dupC]+[3261dupC p.[Phe1088LeufsX5]+[Phe1088LeufsX5	Mother: LS affected siblings-CMMRD	✓	CALMS		T-NHL (2y) ALL (6y)	IgG4 deficiency
P4	8	PMS2 c.ex12 c.2007-2A>G p.Val265_Gln314del	no	no	CALMS		T- NHL (4y)	reduced csBm cells
P5	10	PMS2	no	n.a.	CALMS		Malignant Glioma (10y)	IgG deficiency, low IgA, normal IgM (resembling HIGM)
P6	26	PMS2 c.[2192T>G]+[2192T>G] p.[Leu731Ter]+[Leu731Ter]	Maternal grandfather: CRC (40y)	✓	CALMS	Villous adenoma (small bowel)	CRC (20y) Papilla Vateri Ca (22y) Low grade diffuse astrocytoma (23y) →	reduced csBm cells

							High grade Glioma (26y)*	
P7	21	MLH1 c.[62C>A]+[2146G>A] p.[A21E]+[V716M]	no	no	Cerebral cavernoma Varicosis Vascular malformation CALMS Skin pigmentation - disorder right calve	9 Adenomas (small, large bowel)	T-NHL (1y) B-NHL (15y) Malignant Phylloides tumour (16y) Glioblastoma (21y)	reduced csBm cells, IgG deficiency (IgG2, IgG4 deficiency),low IgM
P8	1	MLH1 c.332C>T p.(Ala111Val)	no	✓	CALMS		T-NHL (8m)	low IgG, IgA, IgM
P9	3	PMS2 c.2007-2A>G	no	✓	CALMS		ALL(2y) Glioblastoma (3y)	IgG deficiency, low IgM
P10	7	MSH6 c.[2653A>T]+[2653A>T] p.[Lys885Ter]+[Lys885Ter]	no	✓	CALMS		Wilms tumour (5y)	-
P11	7	PMS2 c.[2444C>T]+[2444C>T] p.[Ser815Leu]+[Ser815Leu]	no	✓	CALMS		-	IgG4 deficiency