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Germline variants in the mismatch repair genes: Detection and phenotype

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Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy

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ABSTRACT

Constitutional mismatch repair deficiency (CMMRD) is a rare childhood cancer predisposition syndrome caused by biallelic germline mutations in one of four mismatch-repair genes. Besides very high tumour risks, CMMRD phenotypes are often characterised by the presence of signs reminiscent of neurofibromatosis type 1 (NF1). Because NF1 signs may be present prior to tumour onset, CMMRD is a legitimate differential diagnosis in an otherwise healthy child suspected to have NF1/Legius syndrome without a detectable underlying *NF1/SPRED1* germline mutation. However, no guidelines indicate when to counsel and test for CMMRD in this setting. Assuming that CMMRD is rare in these patients and that expected benefits of identifying CMMRD prior to tumour onset should outweigh potential harms associated with CMMRD counselling and testing in this setting, we aimed at elaborating a strategy to preselect, among children suspected to have NF1/Legius syndrome without a causative *NF1/SPRED1* mutation and no overt malignancy, those children who have a higher probability of having CMMRD. At an interdisciplinary workshop, we discussed estimations of the frequency of CMMRD as a differential diagnosis of NF1 and potential benefits and harms of CMMRD counselling and testing in a healthy child with no malignancy. Preselection criteria and strategies for counselling and testing were developed and reviewed in two rounds of critical revisions. existing diagnostic CMMRD criteria were adapted to serve as a guideline as to when to consider CMMRD as differential diagnosis of NF1/Legius syndrome. in addition, counselling and testing strategies are suggested to minimise potential harms.

INTRODUCTION

Constitutional mismatch repair deficiency (CMMRD, MIM #276300) is a rare, autosomal-recessively inherited cancer predisposition syndrome caused by biallelic germline mutations in one of four mismatch repair (MMR) genes (*MLH1*, MIM *120436; *MSH2*, MIM *609309; *MSH6*, MIM *600678; *PMS2*, MIM *600259). CMMRD was first described in 1999 in children of consanguineous parents in Lynch syndrome families.^{1,2} These children, carrying homozygous *MLH1* mutations, developed early onset tumours and presented with a phenotype reminiscent of neurofibromatosis type 1 (NF1) mainly in the form of multiple café-au-lait macules (CALMs). Since these first reports, well over 200 cancer patients with CMMRD have been described. Through these reports and establishment of initiatives, such as the European consortium 'Care for CMMRD' (C4CMMRD), the international biallelic mismatch repair deficiency (BMMRD) consortium and the European Reference Network for rare genetic tumour risk syndromes (ERN-GENTURIS), awareness of CMMRD and our understanding of the phenotype, the pathophysiological mechanisms of tumour development and potential management options have increased substantially.³⁻⁸

Individuals with CMMRD are prone to develop a broad spectrum of tumours. The most common are T-cell non-Hodgkin's lymphomas, high-grade gliomas and colorectal cancers or (advanced)colorectal adenomas, and a number of other malignancies are associated with CMMRD.⁸⁻¹² Although ascertainment bias cannot be excluded, cancer risks appear to be extremely high, as almost all reported patients are diagnosed with a malignancy and approximately 80% of patients develop their first malignancy before the age of 18 years (median age of onset 10 years).^{8-10,13-16} However, attenuated forms of CMMRD with a higher age of tumour onset have also been reported, which are presumably caused by hypomorphic mutations (with reduced penetrance) in at least one allele.¹⁷⁻¹⁹

Already from the first reports, it became clear that the CMMRD phenotype overlaps with that of NF1 and prior to the onset of CMMRD-associated malignancies, it may be indistinguishable from this condition. Multiple (>5) CALMs (>0.5 cm in diameter) are usually the first diagnostic sign of NF1.²⁰ In NF1, CALMs generally already appear in the first year of life, followed by skinfold freckling which is present in most children by school age. Neurofibromas usually develop after puberty and in early adulthood.²⁰ In the past, the majority of NF1 diagnoses were based on clinical criteria from the National Institutes of Health (NIH).²¹ However, in young children who have a de novo *NF1* mutation (accounting for almost 50% of NF1 index cases), the NIH criteria are often not fulfilled. Therefore, many NF1 clinics and paediatricians aim for early diagnosis in

children through genetic testing, made possible by the improved sensitivity of *NF1* mutation analysis protocols.^{22,23}

The most important differential diagnoses of NF1 in children with multiple CALMs are mosaic NF1 and Legius syndrome.^{24,25} From the mutation detection rates in familial and sporadic individuals fulfilling NF1 diagnostic criteria (95% vs 85%),²⁶ it can be deduced that at least 10% of sporadic NF1 cases have mosaic NF1 caused by postzygotic *NF1* mutations that are undetectable in blood lymphocytes. Mosaic NF1 may present as segmental NF1, with NF1 features confined to one part of the body or as a more generalised form that may be indistinguishable from (mild forms) of NF1 due to a germline mutation.²⁵ Legius syndrome (MIM #611431), characterised by CALMs and freckling but absence of other diagnostic NF1 features, is caused by germline mutations in *SPRED1* (MIM *609291).²⁴ About 2.4% of sporadic patients with multiple (>5) CALMs with or without freckling, and in whom no *NF1* mutation can be identified, have Legius syndrome.²⁶ Other potential differential diagnoses of NF1 include Noonan syndrome, Noonan syndrome with multiple lentigines (previously referred to as LEOPARD syndrome), neurofibromatosis type 2 (NF2), Piebald trait and McCune-Albright syndrome.²⁷ However, the latter syndromes are often accompanied by other clinical features that can help in differentiating between syndromes.

Since patients with CMMRD with >5 CALMs and other NF1 signs have been described, it is unsurprising that patients with CMMRD occasionally receive an initial clinical diagnosis of NF1 before receiving the correct diagnosis.^{1,2,28,29} Although not all patients with CMMRD have sufficient CALMs to meet the NF1 diagnostic criterion of >5 CALMs and some reports emphasise that CALMs in patients with CMMRD often differ from the typical uniformly pigmented and smooth-bordered CALMs associated with NF1,³⁰⁻³³ the majority of patients with CMMRD have some hyperpigmented macules reminiscent of NF1-associated CALMs.³⁴ Indeed, Durno et al reported CALMs/hyperpigmented macules in 33 of 34 (97%) patients with CMMRD described by the international BMMRD consortium,¹⁰ and CALMs are present in at least 57 of 76 (75%) patients registered in the C4CMMRD consortium database. The number of CALMs (diameter >1 cm) is known for 35 cases in the latter database, and >5 CALMs >1 cm were found in 26 of 35 (75%) patients (at ages ranging from 0.9 to 21 years) suggesting that at least half of all patients with CMMRD fulfil at least one NIH criterion of NF1 (ie, >5 CALMs).

Awareness that CALMs and occasionally other NF1 signs may be present in a child with CMMRD prior to tumour onset leads to the conclusion that CMMRD is a legitimate differential diagnosis in healthy children with CALMs (with or without other clinical signs of NF1/Legius syndrome) when no causative *NF1* or *SPRED1* mutation is identified,

and no signs of NF1 are found in the parents. Although we can reasonably assume that CMMRD is rare in these patients if the parents are unrelated (see the 'Estimated frequency of CMMRD as a differential diagnosis to NF1 section), a child aged 6 years of consanguineous parents with >5 CALMs and no cancer was recently diagnosed with CMMRD.²⁸ In this situation, a diagnosis of CMMRD may provide an opportunity for cancer surveillance of a highly penetrant childhood cancer syndrome prior to onset of the first malignancy. It will also allow predictive genetic testing and surveillance in relatives at risk for both CMMRD and Lynch syndrome and may impact family planning. However, it is also important to consider the potential harm associated with CMMRD counselling and testing in this setting, and any harm should be outweighed by expected benefits for both the index patient and his/her at-risk relatives. Therefore, physicians and geneticists have begun to discuss if and when to counsel and test for CMMRD in patients suspected to have NF1.³⁵

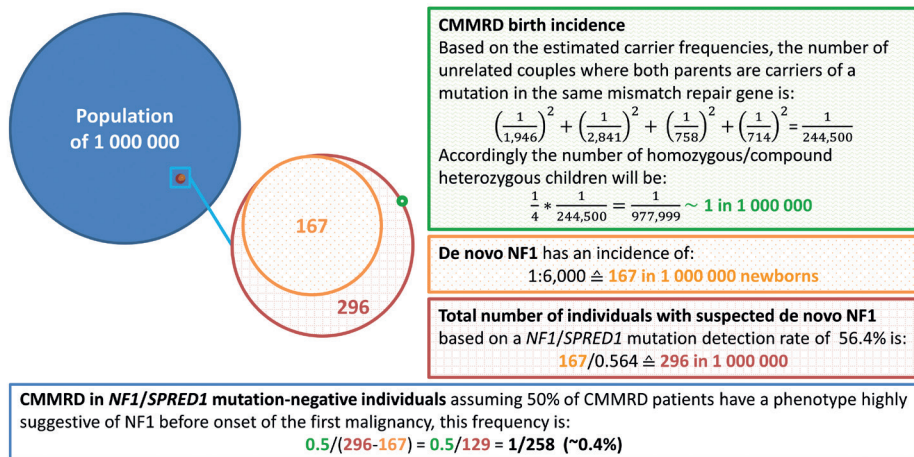
The C4CMMRD consortium, an interdisciplinary team of international experts in the field, has formulated and published diagnostic criteria for the clinical suspicion of CMMRD in patients with cancer,⁸ in addition to surveillance guidelines.⁷ At the most recent workshop in Brussels (26 September 2017), the issue of when to test children without malignancy for CMMRD was addressed by presentations covering four main topics: (i) estimations of frequency of CMMRD as a differential diagnosis of NF1, (ii) potential benefit and harm of CMMRD counselling and testing in a child with no malignancy, (iii) testing prerequisites and strategies to preselect children with a high probability of having CMMRD and (iv) counselling and testing strategies to minimise potential harm of testing. These topics were then discussed among the participants of the workshop. MS and KW summarised the presentations and discussion points in a manuscript draft taking all relevant literature into consideration and citing it as comprehensively and completely as possible. Subsequently, all participants of the workshop who contributed to the discussion and had expertise covering the fields of clinical (onco-)genetics, molecular diagnostics of NF1, Legius syndrome and/or CMMRD, paediatric oncology, (paediatric) gastroenterology and CMMRD surveillance commented and discussed the recommendations in two rounds of revisions until all coauthors consented to the content of the manuscript and proposed adaptation of existing diagnostic criteria to serve as a guideline as to when to consider CMMRD counselling and testing as differential diagnosis for NF1 in children with no malignancy.

ESTIMATED FREQUENCY OF CMMRD AS A DIFFERENTIAL DIAGNOSIS OF NF1

The frequency of CMMRD in children suspected to have NF1 or Legius syndrome, but without a causative *NF1* or *SPRED1* mutation and no overt malignancy, is currently unknown. Since knowledge of disease frequency would help in weighing the possible benefits and harm associated with counselling and genetic testing, we attempt to roughly estimate the frequency.

The incidence of CMMRD in the general population depends on the carrier frequency of MMR mutations. Taking, in contrast to previous lower estimations, all four genes into account, the most recent empiric estimation, based on a large North American/Australian registry, calculated carrier frequencies of 1 in 1946 for *MLH1*, 1 in 2841 for *MSH2*, 1 in 758 for *MSH6* and 1 in 714 for *PMS2* mutations.³⁶ Based on these frequencies, CMMRD incidence was calculated to be about 1:1 000 000 children of unrelated parents (figure 1). The incidence will be substantially higher in populations with founder MMR mutations and in children of consanguineous parents.^{15,37,38}

NF1 is much more common, with an estimated incidence of around 1:2000-1:3000.³⁹⁻⁴¹ Almost half of patients with NF1 are de novo cases.³⁹ To estimate the frequency of patients suspected to have NF1 or Legius syndrome without an *NF1* or *SPRED1* mutation who are actually affected by CMMRD, we took a number of factors into account. In a study using highly sensitive and comprehensive mutation analysis protocols, with mutation detection rates of 96% in patients with familial NF1, *NF1/SPRED1* mutations were identified in 56.4% (764/1354; 751 *NF1* and 13 *SPRED1* mutations) of patients suspected to have sporadic NF1 with >5 CALMs.²⁶ Therefore, based on the incidence of de novo NF1 of 1:6000 newborns and an *NF1/SPRED1* mutation detection rate of 56.4% in patients with >5 CALMs with or without other signs of NF1, we assume that there are 129 patients with >5 CALMs and no *NF1/SPRED1* mutation in a population of 1 million individuals (figure 1). Combining this estimate with the estimated frequency of CMMRD, and assuming that half of all patients with CMMRD present as suspected to have NF1 prior to cancer development, we obtain a figure of 1 patient with CMMRD among 258 children suspected to have NF1 without an *NF1/SPRED1* mutation (ie, ~0.4%) (figure 1). Given this low estimated frequency, a priori chances of diagnosing CMMRD in this group are low.



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Figure 1 Estimated frequency of CMMRD in children suspected to have sporadic NF1/Legius syndrome but without *NF1/SPRED1* mutations and without malignancy. CMMRD, constitutional mismatch repair deficiency; NF1, neurofibromatosis type 1.

POTENTIAL BENEFITS AND HARM OF CMMRD COUNSELLING AND TESTING IN A 'HEALTHY' CHILD

Several factors need to be taken into account when considering CMMRD diagnostics in a child without a (personal history of) malignancy (table 1).

Benefits and their limitations

- i. One of the most important benefits of an early CMMRD diagnosis is the possibility to begin surveillance before cancer development and, consequently, potentially detect cancer at an early stage with better treatment options. With regard to colorectal cancer risk, there is even the opportunity to prevent cancer by removal of intestinal polyps prior to malignant transformation, and existing recommendations for CMMRD surveillance provide clinicians with guidance regarding screening programmes.^{4,6,7} All available guidelines recommend brain MRI, colonoscopies and video capsule endoscopy (VCE) from a young age, as well as gynaecological and urinary tract analysis from age 10 to 20 years. In addition, whole body MRI⁵ and preventive measures such as aspirin intake and/or vaccination with neoantigens^{42,43} are possible modalities that may have a role in CMMRD management. Preliminary analyses in

a small series of patients showed promising results for surveillance measures.⁴⁴ Nevertheless, all recommended programmes are intensive and burdensome and evaluation of the outcome of surveillance protocols in larger studies is yet to be published. Furthermore, when CMMRD is diagnosed in a predictive setting with regard to cancer development, it should be kept in mind that attenuated forms of CMMRD show tumour onset only by the end of the second or in the third decade of life,^{17-19,45} and that no evaluated models are available to accurately estimate penetrance of novel MMR mutations or new combinations of mutations. Hence, it is currently unclear whether a less stringent surveillance programme might be sufficient for a subgroup of patients. Despite these reservations, as sufficient evidence points to an overall high cancer risk, the application of intensive, carefully considered screening recommendations to individuals proven to have CMMRD is justified.

- ii Another advantage of early diagnosis is the possibility to counsel parents regarding the 25% probability that siblings and subsequent children will also be affected, and to discuss the option of prenatal or preimplantation genetic diagnostics while parents are still in the process of family planning. Once again however, informed decision making is complicated by the fact that current estimates of cancer risk are subject to ascertainment bias and individual cancer risks are difficult to predict.

Potential harms

- i Following genetic counselling for CMMRD as a differential diagnosis, parents and children may experience anxiety during genetic testing until the diagnosis is largely excluded. Depending on the diagnostic strategy and performance of the laboratory, this may take several weeks or even months. Moreover, the testing strategy chosen by the laboratory will impact the predictive value of a negative test result (*ie*, the residual risk in the case of a negative test, see the 'Testing strategy' section). This may impact on any remaining anxiety after receiving a negative result. The level of anxiety may also differ depending on the personality and the available coping strategies of the patients/parents and the attitudes of the physicians involved.
- ii Test results definitely confirming or refuting CMMRD will be helpful in the management of the patient and his/her family. However, inconclusive test results will pose a challenge for all parties involved. The most important source of inconclusive results will be variants of unknown significance (VUS) in the MMR genes. Although identification of a VUS is an inherent risk of genetic diagnostics, it is important to minimise the number of VUS and the dilemma with regard to diagnosis and appropriate management of the patient that comes along with it. Therefore, laboratories performing CMMRD analysis in a predictive setting should be prepared

to take any measure necessary to reach a less ambiguous classification of a VUS (C3) as either a (likely) pathogenic (C4/C5) mutation or a (likely) benign (C1/C2) variant.⁴⁶ Tests assessing hallmarks of MMR deficiency in vivo or the effect of the mutation(s) on mismatch repair protein function in vitro will become important in these situations (see the 'Testing strategy' section).

- iii According to Win et al,³⁶ in the general population one in 279 children tested will be heterozygous for an MMR gene mutation. Particularly in the case of a clearly pathogenic *MLH1* or *MSH2* mutation, this results in the unintentional diagnosis of Lynch syndrome in a minor. Lynch syndrome mainly predisposes to adult-onset colorectal cancer and/or endometrial cancer and surveillance only begins around age 20–25 years.^{47,48} Thus, the lack of clinical consequences in children, combined with their right not-to-know, and potential harm due to anxiety and other issues (eg, potential difficulty in acquiring insurance) highlight that a diagnosis of Lynch syndrome is undesirable in a minor.⁴⁹ Further considerations on this topic can be found in the study by Bruwer et al, who offered predictive CMMRD testing to children of parents both carrying familial *MLH1* mutations.⁵⁰ The situation is more complex for *MSH6* and even more so for *PMS2*. Heterozygous mutations in these genes have a 2–4 times higher prevalence,³⁶ but a substantially lower penetrance than *MLH1* and *MSH2* mutations.^{19,51,52} Hence, in an individual lacking a personal or family history of Lynch syndrome-associated cancer, it is uncertain whether the mutation-associated cancer risk is sufficient to diagnose an individual with a cancer predisposition syndrome that warrants intensive cancer surveillance. This concern also raises the question of whether identifying a mutation in an individual without family history for Lynch syndrome justifies predictive genetic testing in parents and other adult at-risk relatives.

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Table 1 Overview of the potential benefits and harms of CMMRD counselling and testing in a child suspected to have sporadic NF1/ Legius syndrome without malignancy and negative outcome of *NF1/SPRED1* germline mutation analysis. CMMRD, constitutional mismatch repair deficiency; VUS, variant of unknown significance.

Potential benefits	Potential harms
<ul style="list-style-type: none"> • Opportunity to begin surveillance before cancer development. • Parents can be informed of the recurrence risk in a sibling/future child. • Lynch syndrome can be diagnosed in family members and surveillance initiated. 	<ul style="list-style-type: none"> • Risks associated with intensive surveillance while efficacy has not yet been evaluated in a large cohort and attenuated forms of CMMRD exist. • Risk of identifying a VUS, resulting in management dilemmas and potentially inducing anxiety. • Risk of diagnosing Lynch syndrome in a minor.

LIMITING POTENTIAL HARM ASSOCIATED WITH CMMRD COUNSELLING AND TESTING IN A CHILD WITHOUT A MALIGNANCY

Assuming that only a small minority (~0.4%) of all *NF1/SPRED1* mutation-negative children from non-related parents will actually have CMMRD syndrome, it would be desirable to reduce the number of individuals/families with whom the possibility of CMMRD needs to be discussed. Therefore, strategies to preselect children with a high probability of having CMMRD are discussed in the following section.

Testing prerequisites

Three prerequisites for considering testing for CMMRD as a differential diagnosis of NF1/Legius syndrome are defined in box 1: (i) the presence of at least one NF1 diagnostic criterion including multiple hyperpigmented skin patches reminiscent of CALMs. The most prevalent NF1 sign present in a patient with CMMRD is hyperpigmented skin patches reminiscent of NF1-associated CALMs and freckling. Other diagnostic

NF1 features such as neurofibromas, Lisch nodules, tibial pseudarthrosis or optic pathway glioma have so far only been seen in patients with CMMRD who also show CALMs.^{1,2,15,53,54} This suggests that CMMRD syndrome is a highly unlikely diagnosis in individuals with only isolated non-pigmentary NF1 features. (ii) *NF1/SPRED1* testing was performed using highly sensitive, comprehensive mutation analysis protocols. The likelihood of identifying CMMRD is of course correlated with the sensitivity of *NF1/SPRED1* mutation analysis performed (further discussed in the 'Testing strategy's section). (iii) The absence of any diagnostic signs of NF1 in either parent. If a parent shows NF1 signs an undetected *NF1/SPRED1* mutation, which might even be present in a mosaic status in the (mildly) affected parent, is probably more likely. NF1 signs might be very subtle in mosaic patients as illustrated by a case of gonosomal mosaicism.⁵⁵ In sign of >5 CALMs. However, because this number of CALMs is present in only a very small percentage of individuals in the general population,⁵⁶ they might be an indication of familial NF1 or at least familial CALMs when present in a parent of a child with clearly >5 CALMs. Therefore, the physician should use his/her clinical experience to interpret the findings in the parent. It is strongly recommended that both parents undergo a full clinical exam for presence of any (mild) features of NF1, and for this purpose a consultation with an ophthalmologist and dermatologist can be considered. It was decided not to include an age limit in the prerequisites for testing, as in CMMRD a wide variability has been observed in the age of cancer diagnosis.^{8,9,17} However, when evaluating a patient who meets the prerequisites it should be kept in mind that the vast majority (around 80%)^{9,10,13-16} of patients with CMMRD will have developed a malignancy or intestinal adenomas by the age of 18 years. Hence, absence of a (pre-) malignancy in an older individual decreases the probability of CMMRD substantially.

Preselection strategies

The presence of one or more additional features suggestive of CMMRD substantially increases the likelihood of this differential diagnosis in a child. The European C4CMMRD consortium has previously defined diagnostic criteria based on features that raise suspicion of CMMRD in a patient with cancer.⁸ By and large, these features could also be used to select children without cancer who have an increased probability of having CMMRD. Therefore, the list of additional features provided in box 1 largely overlaps with the previously defined diagnostic criteria for CMMRD in a patient with cancer (for further details see Wimmer et al⁸).

A feature listed in the original table in the study by Wimmer et al⁸ was 'deficiency/reduced levels of IgG2/4 and/or IgA'. As a recent study on a cohort of 15 consecutive, unrelated patients was unable to show uniform or specific patterns of laboratory

immunological abnormalities,⁵⁷ we did not include this rather unspecific feature in box 1. Two features increasing the likelihood of having CMMRD and not listed in the original table by Wimmer et al⁷ were added to the current table. The first one is a sibling with diagnostic NF1 signs, in the absence of any diagnostic NF1 signs in both parents when gonadal *NF1/SPRED1* mosaicism in a parent has largely been excluded by mutation analysis in the children. Because not all patients with CMMRD have a sufficient number of CALMs to meet the NF1 diagnostic criterion of >5 CALMs, but at the same time presence of 1–3 CALMs is quite common in the general population (20%–1.2%),⁵⁶ we recommend that in this situation >3 CALMs should qualify as an NF1 sign in the sibling. The second new feature is the presence of multiple developmental vascular abnormalities (also known as cerebral venous angiomas) in separate regions of the brain, which were present in 10/10 patients described by Shiran et al,⁵⁸ who suggested this feature as additional non-neoplastic sign indicating CMMRD in a patient with cancer.

Furthermore, a number of patients with CMMRD have been reported to have atypical CALMs with irregular borders and different degrees of pigmentation.³⁰⁻³⁴ Therefore, atypical macules that might be differentiated from typical NF1-associated macules by an experienced clinician/geneticist (see also the ‘Counselling strategy and setting’ section), are suggestive of a differential diagnosis such as CMMRD.³⁰⁻³⁴ Hence, presence of atypical CALMs is also included as an additional feature in box 1.

Some CMMRD-associated features included in box 1 (eg, brain anomalies) will not be detected by routine clinical examination of a patient suspected to have NF1. Since the prevalence and specificity of these features in patients with CMMRD is not well studied, we do not advocate testing for these features unless clinically indicated.

A thorough family history will help in uncovering family members with Lynch syndrome-associated cancers (box 1). When a Lynch syndrome-associated cancer is present, it may be worthwhile, where possible, to analyse the tumour for signs of mismatch repair deficiency.

A thorough assessment of the family history should include also questions regarding consanguinity of the parents. The risk of having CMMRD based on the allele frequencies of MMR gene mutations³⁶ in for example a child of first cousins is $\sim 1/8849$ (using the equation $[p_i f_i] + [p_i^2(1-f_i)] + [p_i f_i + f_i^2(1-f_i)] + [p_k f_i + p_k^2(1-f_i)] + [p_i f_i + p_i^2(1-f_i)]$, where p_i , p_j , p_k and p_l are the allele frequencies of *MLH1*, *MSH2*, *MSH6* and *PMS2* mutations, respectively, and the consanguinity coefficient f_1 for first cousins = $1/16$),⁵⁹ which is about 110 times higher than for a child with unrelated parents.

Box 1 Selection strategy for CMMRD counselling and testing in a child suspected to have NF1/Legius syndrome without malignancy and negative outcome of *NF1/SPRED1* germline mutation analysis

Prerequisites

- Suspicion of NF1 due to the presence of at least one diagnostic NF1 feature¹, including at least two hyperpigmented skin patches reminiscent of CALMs.
- No *NF1* and *SPRED1* germline mutations detected using comprehensive and highly sensitive mutation analysis protocols²
- Absence of diagnostic NF1 sign(s) in both parents[#]

Additional features, at least one (either in the family or in the patient) is required

In the family

- Consanguineous parents.
- Genetic diagnosis of Lynch syndrome in one or both of the parental families.
- Sibling with diagnostic NF1 sign(s)[#]
- A (deceased) sibling³ with any type of childhood malignancy.
- One of the following carcinomas from the Lynch syndrome spectrum⁴: colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer, small bowel cancer, cancer of the bile duct or gall bladder, pancreatic cancer or urothelial cancer before the age of 60 years in first-degree or second-degree relative.

In the patient

- Atypical CALMs (irregular borders and/or pigmentation).
 - Hypopigmented skin patches.
 - One or more pilomatricoma(s) in the patient.
 - Agenesis of the corpus callosum.
 - Non-therapy-induced cavernoma.
 - Multiple developmental vascular abnormalities (also known as cerebral venous angiomas) in separate regions of the brain.
-

¹Neurofibromatosis conference statement.²¹

²See the 'Testing strategy' section.

[#]For further details, please refer to the following sections: 'Testing prerequisites' and 'Preselection strategies'.

³This can be expanded to second-degree and third-degree relatives in populations with a high prevalence of founder mutations.

⁴Møller et al.⁴⁷

CALMs, café-au-lait macules; CMMRD, constitutional mismatch repair deficiency; NF1, neurofibromatosis type 1.

Counselling strategy and setting

Since NF1 is a relatively common and often easily recognisable syndrome for which clear management guidelines exist, many paediatricians order molecular analysis of the *NF1* gene directly without involving a clinical genetics specialist. Counselling and management are more challenging for the much rarer and highly penetrant cancer predisposition syndrome CMMRD. We therefore advocate that predictive (with respect to malignancy) CMMRD testing should be ordered by a physician trained in clinical cancer genetics in a centre with specific expertise in NF1 and related disorders in a multidisciplinary setting. As mentioned above, we suggest that CMMRD does not need to be discussed in all suspected NF1 cases without an identified *NF1/SPRED1* mutation. Following an interdisciplinary discussion and the decision that counselling for CMMRD is indicated in a child without a malignancy, parents and their affected child, depending on his/her age, should be counselled by an experienced geneticist (or, depending on his/her level of education and experience, a genetic counsellor). To be able to make an informed decision on whether they want their child to be tested, parents should be made aware of the potential benefits, with their limitations, and of the various possible outcomes of genetic testing. Nevertheless, considering the low probability of a CMMRD diagnosis, this information should be provided in a way that minimises risk of inducing a disproportionately high level of anxiety. If parents express the need for psychological support or more information on surveillance protocols or cancer treatment options, consultation with a psycho-oncologist or paediatric oncologist should be offered. Specifically trained clinical geneticists/clinicians may be able to differentiate between typical NF1-associated CALMs and the atypical pigmentations sometimes seen in patients with CMMRD.^{30–34} Furthermore, he/she can decide whether another syndrome (eg, Noonan syndrome, Noonan syndrome with multiple lentigines, NF2, Piebald trait and McCune-Albright syndrome) within the differential diagnosis of children with CALMs is more likely and should be addressed by genetic testing prior to CMMRD testing. Lastly, we advise that any centre ordering CMMRD diagnostics is able to facilitate the surveillance programme, either in-house or in cooperating centres within reasonable travelling distance.

Testing strategy

A prerequisite for considering CMMRD counselling and testing as a differential diagnosis in patients suspect for NF1/Legius syndrome is the exclusion of the latter diagnoses with high certainty by absence of germline *NF1/SPRED1* mutations using highly sensitive mutation analyses. The *NF1* gene is large and has a highly diverse mutational spectrum, with private mutations (ie, not reported in any other patient)

identified in a significant percentage of patients (~25%; LM, personal communication). Furthermore, the NF1 mutation spectrum also includes a large proportion of unusual splice mutations that either completely elude genomic DNA (gDNA)-based mutation analysis protocols (eg, deep intronic mutations are found in 2.5%–3% of all patients with NF1) or defy ready classification as (likely) pathogenic mutations without additional transcript analysis (approximately 20% of patients have a splice mutation NOT affecting the AG/GT dinucleotides, but affect coding nucleotides, nucleotides flanking the exons but further upstream/downstream of the AG/ GT dinucleotides or reside very deep into the introns).^{22,60,61} This complicates the classification of novel mutations, especially in the case of silent, missense and intronic variants.⁶² Currently, only comprehensive mutation analysis protocols that include NF1 transcript analysis as a primary or complementary assay, such as direct cDNA sequencing,²³ will achieve sufficient sensitivity to exclude a germline mutation with a 96% certainty.²⁶ Genomic DNA-based mutation analysis methods can achieve high *SPRED1* mutation detection rates (RNA-based mutation analysis performed in >900 patients has not yet identified a *SPRED1* splice mutation that escaped detection in gDNA; LM, unpublished data).

Segmental or mosaic NF1 due to a post-zygotic *NF1* mutation is the most likely differential diagnosis in a child with CALMs, with or without other NF1 signs, and a negative germline *NF1/SPRED1* mutation analysis. Confirming mosaic NF1 however requires identification of the same postzygotic mutation in multiple melanocyte or Schwann cell cultures from biopsied CALMs and neurofibromas, respectively.⁶³ These labour-intensive analyses require specific expertise and therefore are offered only by very few specialised laboratories worldwide. Furthermore, they require invasive procedures. Taken together, this can justify omitting these analyses in children to evaluate mosaic/segmental NF1 prior to CMMRD testing.

In principle, two CMMRD testing strategies can be pursued. The first strategy is direct mutational testing of the MMR genes. The second strategy involves a pre-assay which tests for hallmarks of CMMRD, followed by mutational testing if positive. When opting for direct mutational testing, it should be kept in mind that mutation analysis of *PMS2*, the most commonly mutated gene in CMMRD, is challenging due to the presence of pseudogenes.⁶⁴⁻⁶⁷ Therefore, appropriate methods should be applied to circumvent potential pitfalls of *PMS2* mutation analysis.⁶⁸⁻⁷⁴

An argument in favour of direct mutation analysis using gDNA-based gene panel diagnostics would be that other genes that may mimic the NF1 phenotype (see the 'Introduction' section) can be analysed simultaneously. However, testing a larger number of genes inevitably increases the likelihood of identifying VUS. Therefore, we

advocate a stepwise approach, ruling out other possible differential diagnoses prior to CMMRD testing.

If a VUS is identified in one of the MMR genes, additional analyses should be performed to assist with the interpretation of the variant, such as *ex vivo* functional assays of the mutated gene⁷⁵⁻⁸¹ and/or assays that determine the presence of MMRD in non-neoplastic tissue of the patient. The latter assays could also be used as pre-assays before or in parallel with mutation analysis. This second strategy reduces the risk of VUS identification by providing functional evidence for or against CMMRD, and at the same time increases diagnostic sensitivity by applying two complementary methods.

Microsatellite instability (MSI), defined as a change in the number of mononucleotide or dinucleotide repeats and detectable by alterations in microsatellite fragment length,⁸² is a well-established hallmark of somatic MMRD and is frequently assessed in cancer tissues during testing for Lynch syndrome. MSI is not restricted to neoplastic cells in patients with CMMRD and assays have been developed to detect low levels of MSI in leucocyte DNA of these patients.⁸³ Although highly sensitive and specific in patients with biallelic *PMS2*, *MLH1* and *MSH2* mutations, in patients with biallelic *MSH6* mutations the currently available germline microsatellite instability (gMSI) assays regularly yield normal results.⁸³ This limitation renders this gMSI assay unsuitable for pre-test selection. However, this simple, fast and inexpensive assay can increase diagnostic sensitivity and accuracy by confirming the pathogenicity of *PMS2*, *MLH1* and *MSH2* VUS.²⁸ In the near future, more sensitive, simple and reliable gMSI assays may become available, which could potentially be used for pre-test selection. Recently, a highly sensitive and reliable method for the detection of low levels of MSI was developed, with potential applications in the analysis of MSI in non-neoplastic tissue of patients with CMMRD.⁸⁴ Another assay, which tests for MSI in EBV-immortalised lymphocytes and in parallel for cell tolerance to methylating agents (another functional consequence of CMMRD), has been specifically developed for CMMRD diagnosis.⁸⁵ As this assay is both highly sensitive and specific, it may allow a diagnosis of CMMRD to be definitively confirmed or refuted in cases where mutation analysis and other assays are inconclusive (eg, when only one MMR gene mutation or a homozygous MMR gene VUS has been identified).^{85,86} However, the assay is lengthy, labour intensive and requires expertise, making it ill-suited as a pre-test. Immunohistochemistry (IHC) to detect loss of expression of one or more MMR protein(s) in non-neoplastic tissue, such as small skin biopsies, has also been proposed as a diagnostic assay for CMMRD.^{10,14} However, as taking a skin biopsy is an invasive procedure that can be unpleasant for a young child, IHC should be avoided as a pre-test. Furthermore, IHC may also be insensitive if antigenic but non-functional mutations are present.⁸⁶⁻⁸⁸

Taken together, reliable diagnostics of CMMRD may at times be challenging. Choosing an appropriate testing strategy may depend on the facilities that are most readily available in the centre. Hopefully, more assays will become available that may facilitate simple and reliable selective pretesting for CMMRD.

CONCLUSION

We discussed here the potential benefits and harm (table 1) associated with CMMRD counselling and testing in children suspected to have sporadic NF1/Legius syndrome but without a malignancy and lacking an *NF1* or *SPRED1* germline mutation. After carefully considering all available literature and our own experiences, we arrived at recommendations as to when to counsel and offer CMMRD testing, which are summarised in box 1. We also note that uncertainties exist regarding the incidence of CMMRD and the prevalence of CMMRD-associated features both in the general population and in unselected patients with CMMRD. To evaluate sensitivity and specificity of the proposed selection strategy, it will be important for centres applying these recommendations to systematically record the analysed cases and their outcome. For the evaluation of these prospective data, especially with respect to the sensitivity of the proposed strategy, it will also be important to know the true prevalence of CMMRD among unselected children suspected to have NF1/Legius syndrome, but without a causative *NF1/SPRED1* mutation. Large retrospective studies on anonymised samples are needed to answer this question. Clearly, more data are also needed to further support our recommendations, particularly since published CMMRD cases may be biased towards a more severe phenotype. Therefore, we strongly recommend that the clinical course of all patients with CMMRD who are identified before cancer development is meticulously recorded and submitted to a database. In addition, future studies should also evaluate the psychosocial impact of our recommendations to learn more about the perceived benefits and harms of the strategy proposed. Overall, we believe that with the application of the suggested counselling and testing prerequisites an acceptable balance can be achieved between adequate testing of patients at risk of CMMRD, while avoiding exposing an unnecessarily large number of children and families to any harm that might ensue from counselling and genetic testing for CMMRD.

3

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