Predicting neurofibromatosis type 1 risk among children with isolated café-au-lait macules

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Background: Although isolated cafe-au-lait macules (CALMs) are a common skin finding, they are an early feature of neurofibromatosis type 1 (NF1).

Objective: We sought to develop an algorithm determining the risk of children with CALMs to have constitutional NF1.

Methods: We conducted a retrospective study of patients with isolated CALMs. Diagnosis of NF1 was based on detecting NF1 mutation in blood or fulfilling clinical criteria.

Results: In all, 170 of 419 (41%) and 21 of 86 (24%) children with isolated CALMs who underwent molecular testing and clinical follow-up, respectively, were given a diagnosis of NF1. Presence of fewer than 6 CALMs at presentation or atypical CALMs was associated with not having NF1 ($P < .001$). An algorithm based on age, CALMs number, and presence of atypical macules predicted NF1 in both cohorts. According to the algorithm, children older than 29 months with at least 1 atypical CALM or less than 6 CALMs have a 0.9% (95% confidence interval 0%-2.6%) risk for constitutional NF1 whereas children younger than 29 months with 6 or more CALMs have a high risk (80.4%, 95% confidence interval 74.6%-86.2%).

Limitations: The study was designed to detect constitutional NF1 and not NF1 in mosaic form.

Conclusions: A simple algorithm enables categorization of children with isolated CALMs as being at low or high risk for having NF1. (J Am Acad Dermatol 2017;76:1077-83.)

Key words: algorithm; café-au-lait macules; neurofibromatosis type 1; prediction.

Café-au-lait macules (CALMs) are detected in 2.7% of newborns,[1] and 28% of school-age children.[2] CALMs are multiple (≥3) in about 1% of children,[3] and 14% of adults.[4,5]

CALMs are a hallmark of neurofibromatosis type 1 (NF1) (Mendelian Inheritance in Man no. 162200), which affects 1 in 2000 to 2500 newborns.[6,7] Other

Abbreviations used:

AUC:	area under the curve
CALM:	café-au-lait macule
CI:	confidence interval
NF1:	neurofibromatosis type 1
NIH:	National Institutes of Health

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characteristics of NF1 are skin fold freckling, iris Lisch nodules, neurofibromas, osseous lesions, and tumors such as optic pathway gliomas and malignant peripheral nerve sheath tumors.[8] NF1 is caused by mutations in the NF1 gene.[9,10] Approximately 50% of NF1 cases result from a de novo heterozygous *NF1* gene mutation present in a parental gamete or acquired early in fetal development, whereas the rest are familial.[11,12]

There are well-established criteria for diagnosing NF1, generated by the National Institutes of Health (NIH),[13,14] but the clinical signs appear gradually.[13,15-17] In the absence of a positive family history, young children with NF1 may not have sufficient findings to make a clinical diagnosis.[16]

CALMs are usually the first clinical feature of NF1 to appear. They are sometimes present at birth, but commonly develop between early infancy and ~2 years of age.[18] The presence of 6 or more CALMs, greater than 5 mm before puberty and 15 mm after puberty in largest diameter is a clinical diagnostic criterion for NF1.[14]

CALMs are found in other genetic conditions such as Legius syndrome,[19] described in about 2% of individuals with 6 or more CALMs and negative *NF1* gene testing.[19] Although other conditions such as Noonan syndrome,[20] constitutional mismatch repair deficiency syndrome,[21,22] and ring chromosomes,[23] may be associated with CALMs, CALMs are not a cardinal feature of these conditions.[4]

In the absence of a positive family history, young children with NF1 often lack sufficient criteria to make a clinical diagnosis.[16] We propose a diagnostic algorithm to allow for the categorization of individuals with isolated CALMs as being at low or high risk for having NF1.

CAPSULE SUMMARY

- Isolated café-au-lait macules are ubiquitous, but are also a hallmark of neurofibromatosis type 1.
- The study provides an algorithm enabling categorization of children with café-au-lait macules as being at low or high risk for having constitutional neurofibromatosis type 1.
- Accurate risk assessment may result in better patient care.

METHODS

Patients

This study comprised 2 groups. The first included individuals younger than 18 years with isolated CALMs and a negative family history of NF1 referred for *NF1* gene mutation analysis to the Medical Genomics Laboratory, Department of Genetics, University of Alabama at Birmingham, between 2010 to 2013 (the molecular cohort). Patients with a referral diagnosis of (possible) segmental NF1 were not included. Information on this group included age at time of referral, number (<6 or ≥6) and size (>5 or 15 mm in largest diameter at prepubertal or postpubertal age, respectively) of CALMs, and the presence of CALMs with irregular margins and ragged borders (atypical CALMs). These anonymized data were extracted from a structured requisition form (http://www.uab.edu/medicine/genetics/images/NF1_Test_Requisition_Form.pdf). Patients were given a diagnosis of NF1 based on the presence of a disease-causing *NF1* gene mutation according to the laboratory's criteria. The study was approved by the local institutional review boards.

The second group included individuals with isolated CALMs who were referred to a tertiary care neurofibromatosis referral clinic at Tel-Aviv Medical Center (the clinical cohort). None of the patients in this group had other clinical diagnostic criteria for NF1 (including a family history), or signs of segmental disease. All were examined by a single physician (S. B-S.). The number of CALMs and the presence of atypical CALMs were defined as above. Patients were given a diagnosis of NF1 based on later positive molecular testing, or meeting the NIH NF1 clinical criteria. Non-NF1 CALMs were defined either by negative *NF1* molecular testing in blood or when the clinical criteria for NF1 were not met when the patient was older than 72 months.

Molecular analysis

Blood samples submitted to University of Alabama at Birmingham underwent comprehensive *NF1* gene mutation analysis using an RNA-DNA-based comprehensive approach complemented by DNA-based dosage analyses, as previously described.[24-27] Mutations were classified and annotated following recommendations of the Human Genome Variation Society.

Statistical analysis

Continuous variables were compared using a 2-tailed Student *t* test and logistic regression. Discrete variables were compared using Pearson χ^2 test. A *P* value of less than or equal to .05 was considered significant. Confidence intervals (CI) at the level of 95% were calculated.

A decision tree, using number of sample in the leaf node and pruning algorithm, was used. The data were divided into 3 groups (a cross-validation

Table I. Characteristics of children with isolated café-au-lait macules (N = 505)

Characteristic	Institution	NF1 diagnosis	Non-NF1 CALMs	*P* value
Age at assessment, mean ± SD, mo	UAB	25.6 ± 34.0	70.5 ± 50.4	<.001
	Tel Aviv	14.28 ± 20.16	83.64 ± 48.6	<.001
≥6 CALMs % (No.)	UAB	95.9 (163/170)	71.5 (178/249)	<.001
	Tel Aviv	100 (21/21)	36.9 (24/65)	<.001
Atypical CALMs % (No.)	UAB	3.1 (5/159)	19.0 (47/248)	<.001
	Tel Aviv	0 (0/21)	50.8 (33/65)	<.001

CALMs, Café-au-lait macules; *NF1*, neurofibromatosis type 1; *UAB*, University of Alabama at Birmingham.

method): training and testing groups composed of 80% and 20% randomly selected patients from the molecular cohort, and a validation group (the clinical cohort), respectively. For evaluation of the model, we used the log likelihood and the area under the curve (AUC) based on a receiver operating characteristic. The data were analyzed using software (MATLAB, MathWorks, Natick, MA).

RESULTS

A total of 419 individuals tested at the University of Alabama at Birmingham laboratory fulfilled the research criteria. Mutations in the *NF1* gene were detected in 41% (170 of 419). Patients with *NF1* gene mutations (NF1 group) were younger than the patients without *NF1* mutation (non-NF1 group) at the time of the test (25.6 ± 34 vs 70.5 ± 50.4 months, $P < .0001$). As expected, a lower proportion of patients with NF1 had less than 6 CALMs compared with the non-NF1 CALMs group [4% (7 of 170) vs 28.5% (71 of 249), $P < .0001$]. Patients with NF1 had atypical CALMs 6 times less frequently [3% (5 of 159) vs 19% (47 of 248), $P < .0001$] (Table I).

In all, 21 children in the clinical cohort were given a diagnosis of NF1. A total of 65 children did not fulfill the NF1 diagnostic criteria or had a negative molecular NF1 diagnosis during the follow-up period. In all, 77 children did not meet the clinical criteria, but NF1 status could not be determined as they had not reached the age of 72 months (6 years) or undergone molecular testing.

None of the 21 children given a diagnosis of NF1 had less than 6 CALMs at the first visit compared with 63% (41 of 65) of the non-NF1 CALMs group ($P < .0001$). None of the patients with NF1 had atypical CALMs at the first clinical visit compared with 51% (33 of 65) of the non-NF1 CALMs (Table I).

We generated a decision tree algorithm aimed at predicting individuals with low or high risk of having NF1. The decision tree takes into consideration age, number of CALMs (<6 or ≥6), and presence/absence of atypical CALMs. The error rate (either false positive or false negative) in the training group was 17.7% with an AUC of 0.873, demonstrating a high level of accuracy for the model. Accuracy was maintained for the testing group (error rate of 15.3% and AUC of 0.886). The overall error rate in the molecular group was 17.2%. The error rate for the clinical group was 8.1%, with an AUC of 0.955 (Supplemental Fig 1; available at http://www.jaad.org).

According to the model, children with 6 or more CALMs and younger than 14 months at the time of the molecular test/first clinical analysis had an 88.4% (84/95) and 78.9% (15/19) risk for having NF1 based on molecular/clinical criteria, respectively. Children aged 14 to 29 months who had 6 or more CALMs had 69.2% (45/65) and 80% (4/5) risk for having NF1 in the molecular and clinical cohorts, respectively (Fig 1 and Table II).

In all, 129 of 160 patients in the molecular cohort defined as having a high risk for constitutional NF1 were found to have disease-causing mutations (80.6%, 95% CI 74.4%-86.8%). Nineteen of 24 individuals from the clinical cohort defined as having high risk met the NF1 diagnostic criteria during the follow-up period (79.2%, 95% CI 61.6%-96.7%). Overall, 148 of 184 individuals defined as high risk were given a diagnosis of NF1 (80.4%, 95% CI 74.6%-86.2%) (Table III). The high-risk group included 38% (160 of 419) and 28% (24 of 86) of children in the molecular and clinical cohorts, respectively (Table III).

We identified groups of children who were at low risk for having NF1. None of the 50 children with less than 6 CALMS who were 29 months or older at the time of the molecular diagnosis/initial clinical visit were given a diagnosis of NF1. Similarly, children 29 months or older with atypical CALMs had a very low risk for having molecularly or clinically confirmed NF1: 2.7% (1/37) and 0% (0/28), respectively. Only 1 of 67 individuals from the molecular cohort defined as having a low risk for constitutional NF1 was found to have a pathogenic mutation (1.5%, 95% CI 0%-4.4%); no individual from the clinical cohort defined as having low risk was eventually given a diagnosis of NF1. Thus altogether, only 1 child defined as low risk was given a diagnosis of NF1 (1 of 115,

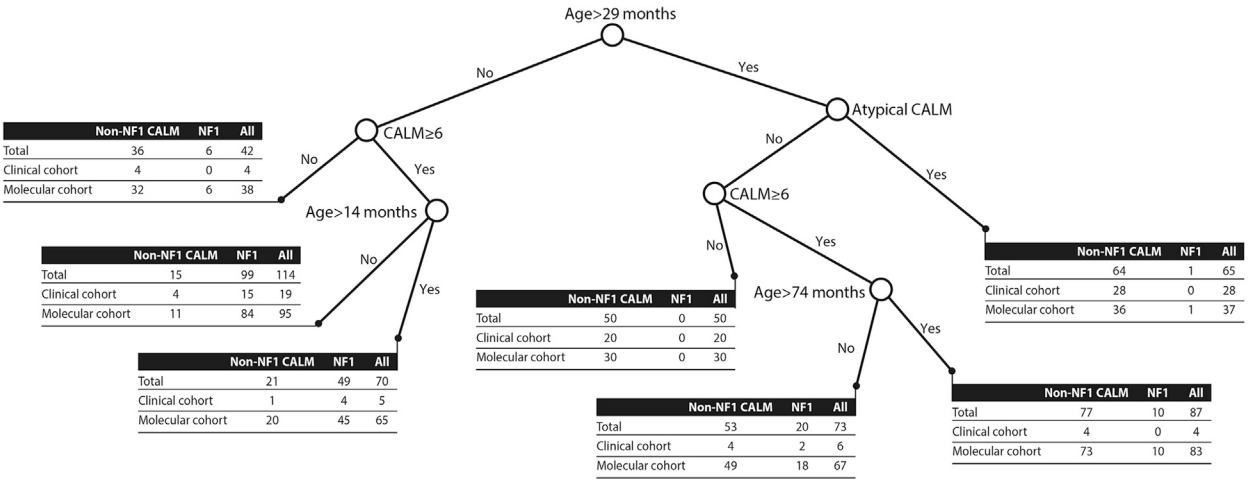

Fig 1. Decision tree for individuals with isolated café-au-lait macules (*CALMs*). The numbers in the tables represent the total number of individuals in the clinical and molecular cohorts along with the total number for each end point. *NF1*, Neurofibromatosis type 1.

Table II. Risk groups for neurofibromatosis 1 diagnosis among individuals with isolated café-au-lait macules

Risk group	Risk—molecular cohort* % (95% confidence interval) [n]	Risk—clinical cohort† % (95% confidence interval) [n]	Risk—combined cohort % (95% confidence interval) [n]
Low risk			
Age >29 mo and <6 CALMs	0 ( ) [0/30]	0 ( ) [0/20]	0 ( ) [0/50]
Age >29 mo and atypical CALMs	2.7 (0-7.9) [1/37]	0 ( ) [0/28]	1.5 (0-4.5) [1/65]
Total	1.5 (0-4.4) [1/67]	0 ( ) [0/48]	0.9 (0-2.6) [1/115]
Intermediate risk			
Age >74 mo and ≥6 CALMs	13.7 (5.8-21.6) [10/73]	0 ( ) [0/4]	13 (5.5-20.5) [10/77]
Age <29 mo and <6 CALMs	15.8 (4.2-27.4) [6/38]	0 ( ) [0/4]	14.3 (3.7-24.9) [6/42]
Age 29-74 mo and ≥6 CALMs	26.9 (16.3-37.5) [18/67]	33.3 (0-71.0) [2/6]	27.4 (17.2-37.6) [20/73]
Total	19.1 (13.3-24.9) [34/178]	14.3 (0-32.6) [2/14]	18.8 (13.2-24.3) [36/192]
High risk			
Age <14 mo and ≥6 CALMs	88.4 (82.0-94.8) [84/95]	78.9 (60.5-97.3) [15/19]	86.8 (80.6-93.0) [99/114]
Age 14-27 mo and ≥6 CALMs	69.2 (58.0-80.4) [45/65]	80.0 (44.9-100) [4/5]	70.0 (59.3-80.7) [49/70]
Total	80.6 (74.4-86.8) [129/160]	79.2 (61.6-96.7) [19/24]	80.4 (74.6-86.2) [148/184]

No values for confidence intervals were provided for groups with a size of 0.
CALMs, Café-au-lait macules.
*Patients tested at the Molecular Laboratory of the University of Alabama at Birmingham.
†Patients tested at the Neurofibromatosis 1 Clinic of the Tel Aviv Medical Center.

0.9%, 95% CI 0%-2.6%) (Table III). The low-risk group included 16% (67 of 419) and 55.8% (48 of 86) of children from the molecular and clinical cohorts, respectively.

Two intermediate-risk groups were detected: children younger than 14 months with less than 6 CALMs, and children older than 29 months with 6 or more CALMs without reported atypical CALMs. These groups had an overall 14.3% and 11.5% risk for having NF1 (Table III).

In total, 54.2% (227/419) and 83.7% (72/86) of the molecular and clinical cohorts, respectively, fulfilled criteria of either high or low risk to have NF1 according to the algorithm at the time of assessment. Of all children referred to the clinic with isolated CALMs (including the 77 not included in the study), 44.2% (72 of 163) could be defined at the initial visit as having either a high or low risk for NF1.

The calculated age cutoffs based on the decision tree algorithm were 14, 29, and 74 months. We investigated if those cutoffs could be replaced by 12, 30, and 72 months, respectively (1, 2.5, and 6 years), to simplify the algorithm for clinical use. The modification had a minimal effect (a prediction value of 79.1% compared with 80% for the high-risk groups, and difference <0.1% for the low-risk groups) (Supplemental Fig 2 and Supplemental Table I; available at http://www.jaad.org).

Table III. Neurofibromatosis type 1 diagnosis among children of the molecular and clinical cohort based on clinical characterizations

Age, mo				CALMs			NF1 diagnosis	
≤14	>14 and ≤29	>29 and ≤74	>74	Atypical	<6	≥6	Molecular cohort	Clinical cohort
+						+	84/95 (88%)	15/19 (79%)
	+					+	45/65 (69%)	4/5 (80%)
+					+		6/38 (16%)	0/4 ( )
		+		+			1/37 (2.7%)	0/28 ( )
		+			+		0/30 ( )	0/20 ( )
		+				+	18/68 (26%)	2/6 (33%)
			+			+	10/83 (12%)	0/4 ( )

CALMs, Café-au-lait macules; *NF1*, neurofibromatosis type 1.

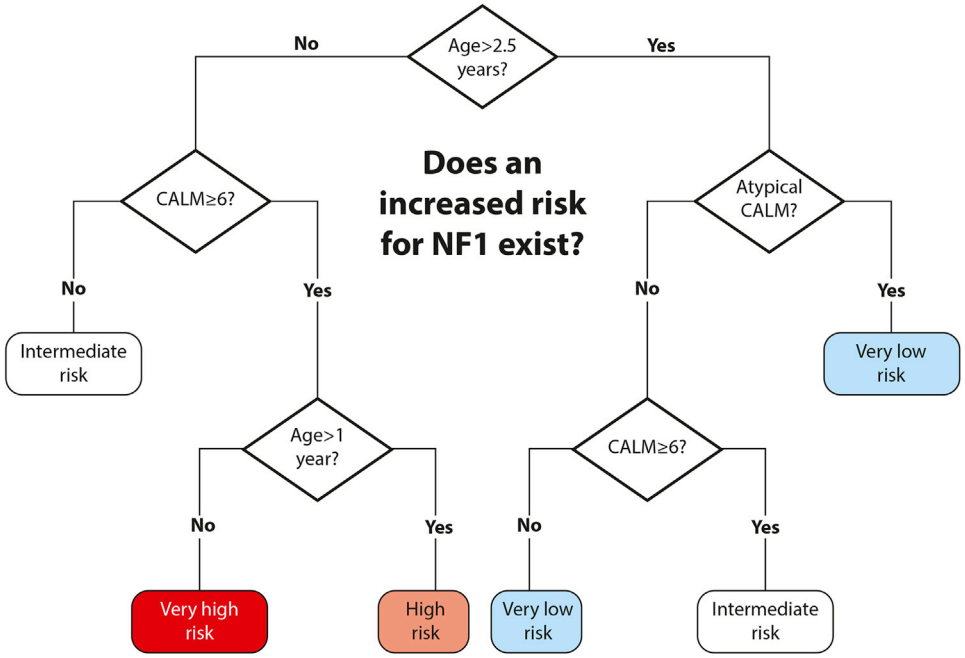

Fig 2. Modified algorithm for detecting risk of neurofibromatosis 1 (*NF1*) among individuals with isolated café-au-lait macules (*CALMs*) based on clinical parameters.

DISCUSSION

Early diagnosis of NF1 in the absence of a positive family history can be challenging. Comprehensive molecular testing of the *NF1* gene is accurate and sensitive for diagnosing NF1, but its use in all individuals with CALMs may be cost-prohibitive and availability varies between countries.[28]

The predictive value of isolated CALMs in NF1 diagnosis has been investigated previously. One study of 110 individuals with isolated CALMs reported that although all patients with an eventual clinical diagnosis of NF1 had at least 6 CALMs, 23% of individuals with 6 or more CALMs at presentation still did not meet the diagnostic criteria by the end of the study. Therefore, the number of CALMs alone was not sufficient for NF1 diagnosis.[29]

We generated a simple algorithm that uses readily available clinical parameters. The algorithm can estimate a child's risk of having NF1, and enables the treating physician to assign individuals with CALMs to 1 of 3 well-defined risk groups at their initial clinical visit (Fig 2 and Table III).

As the less than 1% risk for a child in the low-risk group to develop constitutional NF1 compares with the performance of molecular diagnosis for NF1 (detects disease-causing mutations in greater than 95% of clinically affected individuals),[24-26] it is reasonable not to follow up these children in neurologic/genetic/NF1 clinics.

Children younger than 14 and 29 months with 6 or more CALMs have an 86.8% and an 80.4% risk for having constitutional NF, respectively. We recommend following up these children in the

same way as those meeting full clinical NF1 criteria, unless an alternative diagnosis (eg, Legius syndrome) has been established by molecular testing.

There were some differences between the molecular and the clinical cohorts (Table I). This may be because the clinical group was examined at a NF1 referral clinic, whereas many different specialists performed the clinical analysis for the molecular group. It may also relate to the different methods of diagnosis in the 2 cohorts. Nevertheless, the high level of accuracy of the algorithm for both cohorts demonstrates its applicability for children evaluated by physicians with varying degrees of expertise in NF1.

It is important to note that individuals with isolated CALMs who do not fulfill the NIH diagnostic criteria at the age of 72 months (6 years) may have NF1 because of a milder *NF1* gene mutation such as a missense mutation involving the p.Arg1809 amino acid position,[30] or a specific 3 base pair in-frame deletion in exon 17.[31]

Furthermore, individuals who do not fulfill the diagnostic criteria for NF1 may harbor *somatic NF1* gene mutations (existing in some but not all cells).[25,32] Patients with a somatic *NF1* gene mutation may show a mild or partial disease phenotype, and may not have a detectable *NF1* gene mutation in blood leukocytes. They may potentially have mutations in their gametes,[33] which can result in the birth of affected offspring.[34,35]

It is possible that Next Generation Sequencing techniques may better establish the role and frequency of mosaicism in mild NF1 forms. Individuals with multiple CALMs should receive genetic counseling nearing adulthood to discuss the risk for transmitting NF1 to their offspring.

The proposed algorithm is intended to predict the occurrence of NF1 in its constitutional (nonmosaic), common form. Given the variable phenotype, molecular *NF1* and *SPRED1* analyses are recommended for individuals suspected of having NF1 but with an atypical or milder presentation.

There is a need for large prospective studies of patients with NF1 to re-evaluate the current NF1 diagnostic criteria. These studies might consider clinical criteria related to age at presentation and the addition of diagnostic molecular criteria (including molecular testing of *NF1* and *SPRED1* genes) as in other genetic disorders, such as Marfan syndrome,[36] and tuberous sclerosis.[37]

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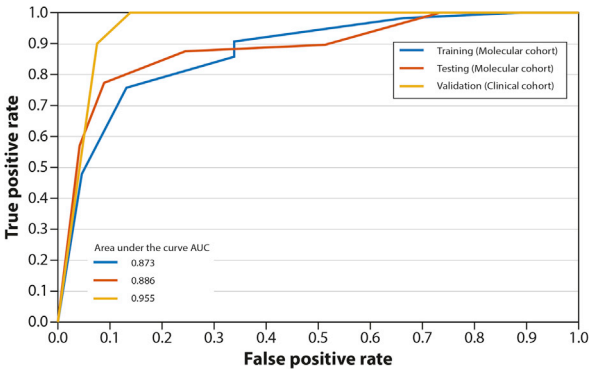
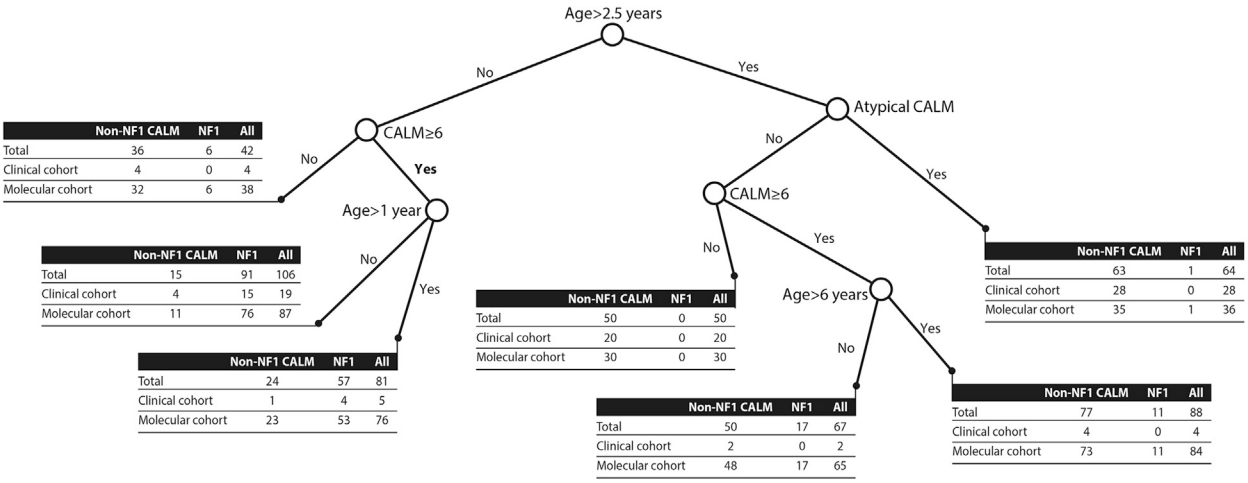


Supplemental Fig 1. Receiver operating characteristic curve of the training and testing groups of the molecular cohort and of the clinical (validation) cohort. The numbers represent the fraction of the area under the curve (*AUC*) for each category.



Supplemental Fig 2. Decision tree for individuals with isolated café-au-lait macules (*CALMs*). Cutoff ages have been modified to simplify the use of the data. The numbers in the tables represent the total number of individuals in the clinical and in the molecular cohort along with the total combined numbers for each end point after age modification. *NF1*, Neurofibromatosis type 1.

Supplemental Table I. Neurofibromatosis type 1 diagnosis among individuals of the molecular and clinical cohort based on clinical characterizations, using simplified cutoffs

Age, y				CALMs			NF1 diagnosis	
≤1	>1 and ≤2.5	>2.5 and ≤6	>6	Atypical	<6	≥6	Molecular cohort	Clinical cohort
+						+	76/87 (87%)	15/19 (79%)
	+					+	53/76 (70%)	4/5 (80%)
+					+		6/38 (16%)	0/4 ( )
		+		+			1/36 (2.8%)	0/28 ( )
		+			+		0/30 ( )	0/20 ( )
		+				+	17/65 (26%)	0/2 ( )
			+			+	11/84 (13%)	0/4 ( )

CALMs, Café-au-lait macules; *NF1*, neurofibromatosis type 1.