# Café-Au-Lait Macules and Intertriginous Freckling in Piebaldism: Clinical Overlap With Neurofibromatosis Type 1 and Legius Syndrome

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Piebaldism is an autosomal dominant disorder characterized by congenital hypopigmented patches of skin and hair and has been found to be associated with mutations in the *KIT* or *SLUG* genes. Café-au-lait macules (CALM) may occasionally be seen in piebaldism. There are four reports describing six patients who were said to have both piebaldism and neurofibromatosis type 1 (NF1) due to the presence of multiple CALM and intertriginous freckling, but none of these patients had undergone comprehensive *NF1* mutation analysis. We describe a large family with piebaldism in which two members meet diagnostic criteria for NF1 based on the presence of >5 CALM and intertriginous freckling. Interestingly, only these two family members are of mixed race, which could be of importance. A novel complex mutation in the *KIT* gene was identified in several family members affected with piebaldism; the proband meeting diagnostic criteria for NF1 also underwent comprehensive *NF1* and *SPRED1* testing with no mutations detected. These findings suggest that piebaldism may occasionally include CALM and intertriginous freckling, which may create diagnostic confusion especially in the absence of a family history of piebaldism. However, careful clinical evaluation and molecular testing if necessary should distinguish these two disorders. © 2012 Wiley Periodicals, Inc.

**Key words:** café-au-lait macules; piebaldism; *KIT* gene; *SLUG* gene; neurofibromatosis; Legius syndrome; *SPRED*1 gene

## INTRODUCTION

Piebaldism is an autosomal dominant disorder characterized by congenital hypopigmented patches of skin and hair. This pattern of hypopigmentation typically occurs on the ventral trunk, extremities, forehead, and eyebrows, along with a white forelock in most patients. Biopsies of these areas typically reveal absence of melanocytes. Piebaldism is caused by inactivating mutations or deletions in the *KIT* proto-oncogene [Giebel and Spritz, 1991] or uncommonly by deletions in the *SLUG* gene [Sánchez-Martin et al., 2003]. Neurofibromatosis type 1(NF1), characterized by multiple café-au-lait macules (CALM), intertriginous freckling, neurofibromas, Lisch

nodules, and other features, is known to be caused by mutations in the *NF1* gene [Viskochil et al., 1993]. The recently delineated Legius syndrome, caused by loss-of-function mutations in the *SPRED1* gene, is characterized by multiple CALM and intertriginous freckling without neurofibromas, eye findings, or bone abnormalities typical of NF1 [Brems et al., 2007]. Other features may include lipomas, macrocephaly, and learning disabilities.

There are four reports, all in the dermatology literature [Chang et al., 1993; Tay, 1998; Angelo et al., 2001; Duarte et al., 2010], of six patients who were said to have both piebaldism and NF1 based on hypopigmented lesions as well as CALM and intertriginous freckling. Another report [Sánchez-Martin et al., 2003] describes two patients with piebaldism associated with *SLUG* mutations in which the diagnosis of NF1 was considered because of CALM and intertriginous freckling. Overlap of clinical features in these two disorders can lead to diagnostic confusion. Importantly, none of the former reports included comprehensive *NF1* mutation analysis and hence the assumed diagnosis of NF1 was not confirmed at the genetic level. We recently evaluated a large family with piebaldism in which two members also fulfilled NIH diagnostic criteria for NF1 and we evaluated them from a molecular standpoint.

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## CLINICAL REPORTS

Patient 1 (III.2) is an 8-year-old boy who was referred to the genetics clinic for possible NF1 and Waardenburg syndrome. He was born to a 37-year-old white mother and a 40-year-old African-American father after a pregnancy complicated by preeclampsia. Birth weight at 36 weeks gestation was 2,440 g (25−50%). At birth, he was noted to have a white forelock and extensive hypopigmentation of the skin on the abdomen and legs. He gradually developed CALM and his mother was told he might have NF1. Hearing evaluation was normal. He had two eye exams, which were normal. At 5 years of age, he was diagnosed with a dorsal exophytic brainstem tumor, thought to be a low-grade glioma (Fig. 1). This tumor has not required surgery, radiation, or chemotherapy. He also had a congenital hairy nevus removed from the wrist. His developmental milestones were normal. Initially his grades in school were good, but recently, he began struggling. Neuropsychological evaluation suggested ADHD and poor executive function.

At age 9 and 8/12 years, his height was 140 cm (50−75%), weight 31.5 kg (50%), and head circumference 54 cm (75%). He had a white forelock and a few white hairs in the occipital region. There were a few white eyebrow hairs and hypopigmentation of the forehead, chest, and anterior and posterior knees. He developed some islands of pigmentation within these areas over time (Fig. 2a−c). He also had >5 CALM at least 0.5 cm in size and axillary and inguinal freckling (Fig. 3a,b). He had no visible Lisch nodules, heterochromia, or neurofibromas. The remainder of his exam was normal.

Patient 2 (III.3) is the 22-year-old maternal half-brother of Patient 1. He is also of mixed race and was born with a white forelock and extensive hypopigmentation of the skin. He has developed some islands of pigmentation in some of the hypopigmented areas. In addition, he has >5 CALM at least 1.5 cm in size and intertriginous freckling but no neurofibromas (Fig. 4a−d).
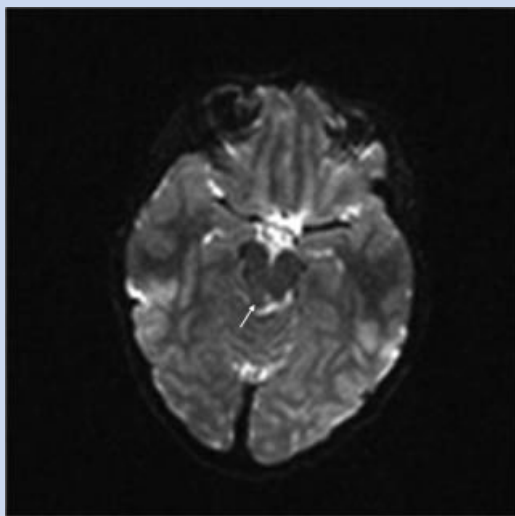


FIG. 1. Brainstem tumor in Patient 1.

The 49-year-old mother of Patients 1 and 2 (II.2) had a white forelock at birth and her hair became all white between 25 and 30 years of age. She also has white spots on her arms, legs, and abdomen but no CALM or intertriginous freckling.

Two maternal half-sisters of the proband (III.4 and III.5) have white spots on the skin and a history of a white forelock. Neither of these sisters has CALM or intertriginous freckling and neither is of mixed race.

## METHODS

Genomic DNA was extracted according to the conditions recommended by the manufacturer. Genomic DNA from whole blood was isolated using the BioRobot EZ1 workstation (Qiagen, Valencia, CA). Saliva DNA was isolated using the Oragene DNA kits (DNA Genotek, Ottawa, Canada). PCR primers were designed by using thePrimer3 program (http://fokker.wi.mit.edu/primer3/input.htm). The reference sequence of *KIT* was from NG_007456.1 RefSeqGene. Mutation analysis was performed by PCR amplification and bi-directional DNA sequencing of all coding exons and all exon/intron borders (at least 50 nt into the intron). PCR was performed using the KAPA2G Robust HotStart following manufacturer recommendations and the conditions recommended by the manufacturer (KAPA Biosystems, Woburn, MA). The samples were processed through 95°C for 12 min, 5 cycles of 60−55°C for 40 sec, 72°C for 1 min, 95°C for 30 sec, 30 cycles of 94°C for 30 sec, 55°C for 40 sec, 72°C for 1 min with a final extension step of 72°C for 5 min. Sequence analysis was performed using Mutation Surveyor program (SOFTGENETICS; http://www.softgenetics.com/mutationSurveyor.html). Mutation analysis was performed by using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/).
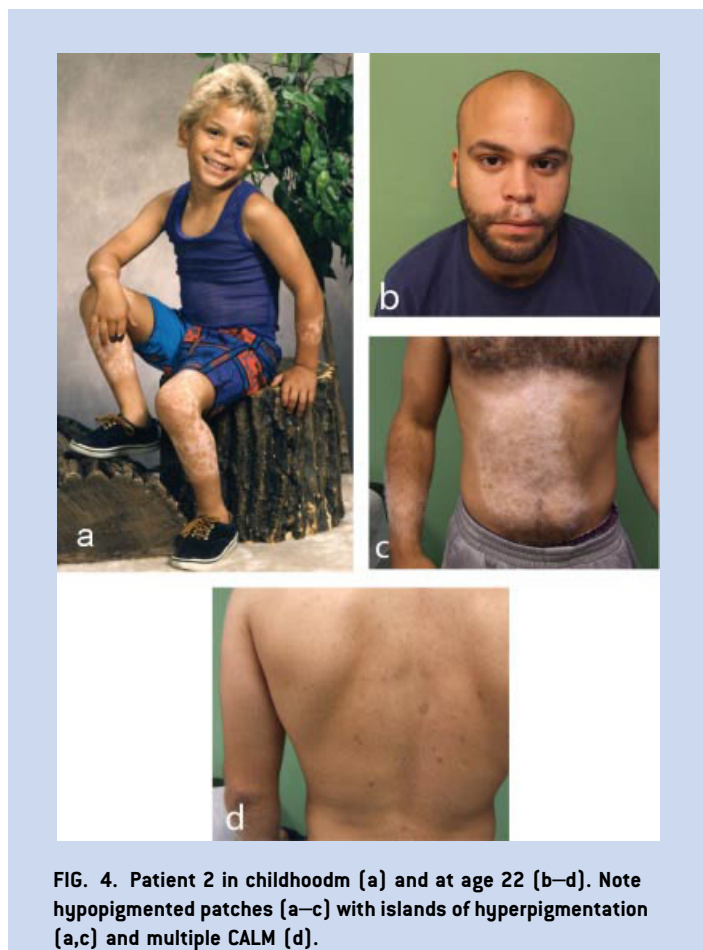
The primary assay for comprehensive *NF1* and *SPRED1* mutation analysis is a direct cDNA sequencing approach based on the amplification of the entire *NF1* and *SPRED1* coding regions in three (*NF1*), respectively, one (*SPRED1*) RT-PCR fragments and subsequent sequencing of the entire PCR products with internal primers. Details on the RT-PCR reactions and primers can be found in Messiaen and Wimmer [2011] and Spencer et al. [2011]. To avoid illegitimate splicing, known to lead to multiple aberrant splice variants that impede the detection of mutations in an RNA-based approach [Messiaen et al., 2000], total RNA is extracted from phytohemagglutinin (PHA)-stimulated short-term lymphocyte cultures treated with 200 μg/ml puromycin for 4 hr prior to cell harvest to prevent the nonsense-mediated RNA decay. BigDye Terminator Cycle Sequencing chemistry was used for sequencing (Applied Biosystems, Foster City, CA). The sequencing reactions were subsequently run on an automated capillary sequencer and analyzed using the sequence analysis program SeqScape V2.5 (Applied Biosystems) and SequencePilot (JSI Medical Systems, Kippenheim, Germany). *NF1* nucleotide numbering is based on GenBank reference sequence NM 000267.3; *SPRED1* nucleotide numbering is based on GenBank reference sequence NM 152594.2. These studies were complemented with dosage analysis approaches (MLPA) to detect deletions/duplications extending beyond the borders of the RT-PCR products. MLPA was performed using probe mixes P295 (*SPRED1*), P122

FIG. 2. Patient 1 at age 9 years. Note hypopigmented patches of hair and skin (a–c) and CALM on chest (b).

(NF1; MRC-Holland, Amsterdam, The Netherlands), following the manufacturer's instructions.

## RESULTS

Sequencing of the KIT gene in patient 1 showed two base substitutions c.[2021G>A;2024A>C] resulting in amino acid substitutions p.[Cys674Tyr;Tyr675Ser], which were predicted to be damaging. This prediction is based on the compound mutation segregating with the piebaldism in the family as well as information from PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org). Both changes affect highly evolutionarily conserved amino acids. Comprehensive NF1 and SPRED1 mutation analysis by sequencing all exons and dosage analysis did not reveal any mutations in these genes in Patient 1 (III.2). The compound KIT mutation was found in Patients II.2, III.2, III.3, and in three additional family members as illustrated in Figure 5. Four unaffected family members were studied and found not to have the KIT mutation. Therefore, c.[2021G>A;2024A>C] seems to represent a de novo mutation in Patient II.2.



FIG. 3. Patient 1. Note CALM (a) and axillary freckling (b).



FIG. 4. Patient 2 in childhoodm (a) and at age 22 (b–d). Note hypopigmented patches (a–c) with islands of hyperpigmentation (a,c) and multiple CALM (d).

## DISCUSSION

Review of the literature revealed four reports, all in the dermatology literature, of six patients who were said to have both piebaldism and NF1 [Chang et al., 1993; Tay, 1998; Angelo et al., 2001; Duarte et al., 2010]. Chang et al. [1993] reported a 17-year-old Yemenite male with a white forelock and eyebrows and white patches of skin. He also had six CALM and bilateral axillary freckling. Chang et al. [1993] also reported a 7-year-old black boy with white forelock and eyebrows, white skin patches, numerous CALM, and bilateral axillary freckling. Tay [1998] reported a 15-year-old Chinese girl with a white forelock and white skin patches, numerous CALM, axillary and inguinal freckles, and Lisch nodules. Her mother and maternal grandfather were said to have the same findings. Angelo et al., [2001] reported an 11-year-old girl of Cape Verdian descent with a white forelock, white skin patches, multiple CALM, axillary and inguinal freckling, and no Lisch nodules. In none of the aforementioned studies were mutational analyses for NF1 and KIT performed. Duarte et al., [2010] reported a 3-year-old boy with a white forelock, white skin patches, CALM, and intertriginous freckling. His father had similar findings. Both were found to have a KIT mutation but no mutation in the NF1 gene was found. Unfortunately, no details were provided as to how the NF1 mutational analysis was performed so it is unclear what the sensitivity of the testing was. Sánchez-Martín et al. [2003] studied 17 patients with piebaldism who did not have KIT mutations. Three
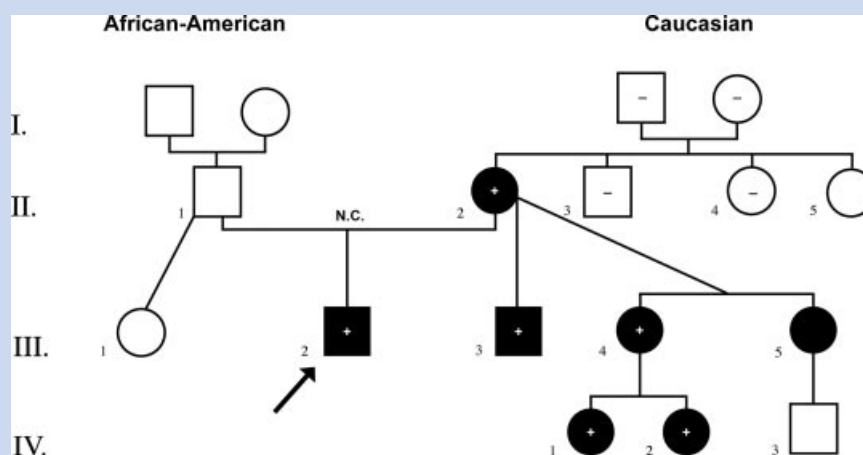
FIG. 5. Pedigree of family. (+) In family members with the KIT mutation and (−) in those found not to have the KIT mutation.

of these 17 were found to have deletions of the *SLUG* gene. Two of these patients, who were Caucasian, were also noted to have CALM and intertriginous freckling; the diagnosis of NF1 was considered in these two patients.

There is a complex network of interacting genes that regulate the embryonic development of melanocytes from neural crest cells. Piebaldism, which is due to a defect of migration of neural crest-derived melanoblasts to the skin, closely resembles the dominant white spotting disorder of mice with mutations in either the *kit* or the *slug* gene [Chabot et al., 1988; Geissler et al., 1988; Pérez-Losada et al., 2002]. The clinical features of piebaldism may overlap those of other human disorders with pigmentary abnormalities, such as Waardenburg syndrome, vitiligo, Ziprkowski syndrome, and rarely neurofibromatosis. Multiple CALM may also be seen in several other disorders, such as Bannayan, Noonan, cardio-facial-cutaneous, Silver–Russell, McCune–Albright, Bloom, multiple lentigenes syndrome, tuberous sclerosis, and ataxia-telangiectasia.

Although our two patients with piebaldism and those in the literature meet the NIH diagnostic criteria for NF1 based on multiple CALM and intertriginous freckling [NIH Consensus Development Conference, 1988], one can distinguish both disorders clinically. The predominant feature of piebaldism is hypopigmented areas of the skin characterized by absence of melanocytes. NF1 is primarily characterized by hyperpigmentation with CALM, which have an increased number of melanocytes and macromelanosomes. None of the patients with features of both piebaldism and NF1 has developed other features of NF1, such as neurofibromas or optic gliomas, although the patient reported by Tay [1998] was said to have Lisch nodules. In a sporadic patient with the constellation of pigmentary signs as found in the two patients described here, a correct diagnosis might however be difficult solely based on clinical signs. Indeed, as NF1 is a relatively frequent genetic disorder affecting 1/3,000 individuals worldwide, occasionally patients have been described having a distinct genetic disorder as well as NF1 [Thiel et al., 2009; Basaran et al., 2010]. Molecular studies can help with the diagnosis in such patients.

Of note is that the two patients in our family with piebaldism who also had CALM and freckling are of mixed race. It is possible that this contributes to their pigmentary findings. It is known that melanosomes, which extend throughout the epidermis [Montagna and Carlisle, 1991], are larger and more numerous in black skin than white skin. In white skin, typically only a few melanosomes are present in a sparse distribution, primarily in the basal keratinocytes. However, none of the patients with piebaldism, CALM, and freckles reported in the literature were said to be of mixed race and were from a variety of ethnic backgrounds.

Piebaldism and NF1 result from mutations in different genes. Piebaldism results from mutations in the *KIT* gene most commonly or occasionally in the *SLUG* gene. Mutations in the *KIT* gene result in decreased receptor tyrosine kinase signaling, impaired migration of melanoblasts to the skin during embryologic development, and a decrease in melanogenesis [Spritz et al., 2004]. *SLUG*, a downstream target of the *KIT* signaling pathway, is also responsible for piebaldism in some patients [Sánchez-Martin et al., 2003]. On the other hand, mutations or deletions in the *NF1* gene result in hyperactivation of the *Ras* proto-oncogene and enhanced receptor tyrosine kinase signaling.

Our proband also has a brainstem tumor thought to be a low-grade glioma. Mutations in the *KIT* proto-oncogene which activate the receptor have been reported in mast cell leukemia lines, gastrointestinal stromal tumors, acute myeloid leukemia, seminomas, and dysgerminomas. Gatto et al. [1985] reported a large family with piebaldism in which six members also had various types of cancers including one adult with an astrocytoma. Gomes et al. [2007] demonstrated *KIT* overexpression in various types of malignant gliomas. Interestingly, brain stem gliomas are common in NF1. However, since the *KIT* mutations in piebaldism are inactivating mutations, it is difficult to explain both piebaldism and tumors with the same mutation. Therefore, it is uncertain whether our proband's *KIT* mutation is related to his brainstem tumor.

In summary, we conclude that our family and most likely those described in the literature have piebaldism, not NF1 or Legius syndrome. The presence of CALM and intertriginous freckling in

these patients suggests that these findings are occasional features of piebaldism, possibly more likely to occur in individuals of mixed race. However, at this point, the mechanism of CALM development in piebaldism remains unknown. Careful clinical evaluation of patients with these overlapping features as well as molecular studies in some situations should readily distinguish these two disorders.

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