Gene discovery in extensive dermal melanocytosis reveals multiple mosaic causes

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Acknowledgements: We gratefully acknowledge the participation of all patients in this study, research coordination by Jane White and recruitment by Eglantina Marku.

Funding sources: VK and NK are funded by the National Institute of Health and Social Care Research (NIHR) UK award number NIHR300774.

Conflicts of interest: The authors declare no conflicts of interest.

Data availability: All relevant data are available within the submission.

Ethics statement: The study was approved by the London Bloomsbury Research Ethics Committee as part of the Great Ormond St Hospital Rare Dermatology Diseases Resource (12/LO/1522, 17/LO/1783, 22/LO/0848).

Patient consent: The authors attest to obtaining written patient/parent/legal guardian consent for all patient photographs or other identifiable material, with the understanding that this information may be publicly available.

What is already known about this topic?

- Congenital Extensive or Atypical Dermal Melanocytosis (EDM) must be differentiated from common transient "blue spots", as it is a mosaic disorder with risk of involvement of other organs and of melanoma development
- Mosaic variants in one gene *GNAQ* have previously been associated with EDM in a handful of cases
- Periocular cutaneous involvement can be associated with ocular involvement
- EDM is thought to be commoner in darker skin tones

What does this study add?

- We identify here four new genetic causes of congenital EDM: mosaic pathogenic variants in genes *HRAS*, *PIK3CA*, *ACTB* and *GNA11*
- Ophthalmological involvement is common, found in 40% of this cohort, and is not exclusive to patients with periocular cutaneous involvement
- Two patients had evidence of ischaemic infarcts on brain MRI

- It is possible that EDM is simply more visible in darker skin tones rather than more common
- EDM includes the previous diagnoses of naevus of Ota or Ito

What are the clinical implications of this work?

- Genotyping may help stratification of melanoma risk in the future, as some causative genes are more commonly associated with sporadic melanoma than others
- All patients with EDM should have an ophthalmological assessment to establish the full congenital phenotype, even if there is no periocular skin involvement
- Neurological symptoms and signs should be sought and if present lead to MRI of the CNS
- Clinicians should be aware that EDM may be difficult to detect in lighter skin tones

Abstract

Background Extensive and/or atypical dermal melanocytosis (EDM) is abnormal macular blue/black skin pigmentation which can be associated with other congenital abnormalities and an increased risk of melanoma. It is likely underdiagnosed due to phenotypic overlap with common transient dermal melanocytosis (so-called "blue spots"), and is frequently misdiagnosed as congenital melanocytic naevi. The genetic cause has remained largely unknown, with mosaic variants in one gene *GNAQ* described in only a handful of cases to date.

Methods Forty-seven patients with EDM only were recruited for phenotypic and targeted deep next generation sequencing of affected skin. Ophthalmological examination was performed routinely, and CNS MRI performed in five patients where there was clinical suspicion.

Results Ophthalmological involvement was seen in 40%, and importantly was not restricted to those with periocular cutaneous involvement. Two patients of five scanned had evidence of ischaemic infarcts. Mosaic causes of EDM were found in four new genes *HRAS, ACTB*, *PIK3CA*,

and *GNA11* alongside further cases of *GNAQ*. The *HRAS* variant has previously been reported in the germline.

Conclusions Clinically, our findings highlight the importance of differentiating EDM from common blue spots, and we recommend ophthalmological investigation even in the absence of periocular cutaneous involvement. The association with CNS infarct is unclear, but we suggest clinical neurological features be sought and MRI undertaken if there are concerns. Genetically, these results not only expand the causative genotype of EDM, but also challenge our concept of the Mosaic Disorders currently described with *HRAS, ACTB*, *PIK3CA*, and *GNA11*. Stratification by genotype may help determine patient melanoma risk more accurately in the future. Patient- and variant-specific genetic counselling should be given where there is a potential risk of germline transmission to offspring.

Extensive and/or atypical dermal melanocytosis (EDM) is a congenital cutaneous pigmentary disorder characterised by deep blue or grey macular skin lesions, which can be single or multiple, and can vary from a few centimetres in diameter to covering most of the body surface area. These vary in colour intensity both between individuals and between different areas in one individual, with generally more distinct edges associated with increased colour intensity. On the basis of natural history studies EDM is currently defined as dermal melanocytosis fulfilling any two of the following criteria: (i) involvement of sites other than only the lumbosacral area, (ii) persistence beyond the first two years of life, (iii) areas > 10 cm in diameter at birth and (iv) some areas of accentuated deep pigmentation with clearly defined borders[1]. This definition helps to differentiate EDM clinically from so-called "blue spots", very common birthmarks which vary in incidence between ancestral populations, from 95% in African neonates to 10% in white Caucasians. These appear as blue-greyish flat lesions with indistinct edges overlying the buttocks and lower back, and undergo spontaneous resolution over the first few years of life[2-4]. It is likely

that the common nature of "blue spots" has reduced the recognition of EDM as a disease entity[5-8]. This definition of EDM includes the older diagnoses of naevus of Ota and naevus of Ito, defined as dermal melanocytosis in the periocular and shoulder region respectively. It is not known if the location of EDM on face or neck is any commoner than elsewhere on the body, or whether they were labelled as separate entities for other reasons such as visibility or being sufficiently far from the lumbosacral area to be considered more clearly abnormal.

EDM has been associated with a long list of extracutaneous complications. The commonest complications are glaucoma[9] and leptomeningeal/orbital melanocytomas[10-12] but also described are colocalized cleft lip[13], café-au-lait pigmentation, mycosis fungoides and dermatofibrosarcoma protuberans[1,14,15]. Notably, EDM is also associated with an increased risk of melanoma development[10,16,17]. This has so far been most commonly documented in the context of patients with naevus of Ota, in which malignant transformation has so far most commonly involved the uveal tract, orbit and meninges, more rarely the affected skin[16]. Melanoma usually presents in late adulthood with a higher risk in individuals with White Caucasian ancestry, and carries a very poor prognosis with early metastatic disease[16,18]. However melanoma has also been described in the central nervous system and subcutaneous tissue in the paediatric population, and in all cases with a rapidly fatal outcome[19,20].

The genetics of EDM were first studied in 2016, when mosaic variants in gene *GNAQ* were identified in two patients, one affecting codon 183 and one 209[14]. In retrospect a *GNAQ* variant had been described in a single case of naevus of Ota as part of a study of blue naevi[21]. In the

2016 study, *GNAQ* variants only explained 20% of the initial cohort of 11 patients however, with the others being wildtype for *GNAQ* and *GNA11*.

We sought to gain a better understanding of the clinical spectrum and causative genetics of this disease and potentially to stratify patients for prospective studies of melanoma development.

Methods

EDM was defined as above and as previously[1]. None of the patients included in this cohort had any vascular lesions (hence they did not have PPV), or an acquired progressive pattern of lesions, or other findings suspicious for metabolic storage disorders. A cohort of 47 patients were recruited from the NHS England Rare Disease Clinical Network for Mosaic Disorders at Great Ormond St Hospital for Children, London. Recruitment to the study was offered to all patients seen consecutively in clinic over the period 2007-2024. The study was approved by the London Bloomsbury Research Ethics Committee (12/LO/1522, 17/LO/1783, 22/LO/0848). Patients were followed up as part of standard clinical practice with a mean and median age at last follow-up of 5.1 (SD 3.9) and 4.4 years, respectively (range 0.5 -16.7 years).

Phenotypic/epidemiologic variables collected were age, sex, self-designated ethnicity, phenotype of skin lesions and presence and phenotype of non-cutaneous abnormalities. All patients were referred for an ophthalmological evaluation to look for the presence of anterior and posterior eye pigmentation abnormalities and intraocular pressure. A history of neurological problems was sought directly, and in those with significant symptoms or signs, a brain MRI was recommended.

Phenotypic data were analysed using SPSS version 30.0.0.0 (171). Punch skin biopsies from affected skin (3mm for single lesions on the face, 4mm elsewhere) were offered to all and performed in 35 patients after written informed consent from parents. DNA was extracted from whole skin for genetic studies and routine histological examination was performed on the same biopsy. A blood sample for DNA extraction was obtained in 22 patients, to allow comparison with affected tissue.

In two patients, a mosaic pathogenic variant in *GNAQ* c.548G>A, p.(Arg183Gln) or *GNAQ* c.626A>C, p.(Gln209Pro) had already been identified as previously published[14]. The remaining 33 patients had skin DNA sequenced on targeted next generation sequencing panel R327 (mosaic disorders, UK National Genomic Test Directory) in the diagnostic laboratory as part of routine clinical care. Sequencing was via custom TWIST target enrichment and Illumina NextSeq 500/550 paired-end 75bp reads. The Illumina Dragen platform was used for alignment, and an in-house custom pipeline (MosaicMiner) for variant calling and annotation. The minimum coverage threshold for the panel was 700X and variant allele frequency (VAF) detection limit given as 1%. Secondary validations of all positive results were performed via standard methods for digital droplet PCR or Sanger sequencing in the diagnostic laboratory. Where results of the targeted panel were negative and where sufficient skin DNA remained, whole exome sequencing was performed via the research laboratory on thirteen samples. Whole exome sequencing (NovaSeqX 10B 300 cycles) using Twist Human Exome (NEB library preparation) in blood (50X coverage) and skin samples (1000X coverage) was performed to identify germline and somatic variants using a custom designed in-house pipeline. Briefly, reads were mapped to the GRCh38 reference using Burrow Wheel Aligner, and BAM files were generated after duplicate marking (GATK

MarkDuplicates) and base recalibration (GATK BaseRecalibrator and GATK Apply BQSR). VCF files containing germline and somatic variants were created using either HaplotypeCaller or Mutect2 respectively. Resulting VCF files containing variants were analysed using Clinical Insight (QCI) Interpret (QIAGEN) and selected variants were manually inspected using IGV 2.16.2 (examples Supplementary Figure 1). New potential mosaic candidate variants were validated via a second method in the diagnostic laboratory.

Results

Cohort demographics

Forty-seven patients with EDM were recruited, of which 32 were female (68%). Mean and median ages at first evaluation were 2.2 (SD 2.9) and 1.0 years respectively (range 0.1-14.8). Mean and median age at most recent evaluation for routine follow up were 5.1 (SD 3.9) and 4.4 (range 0.5 - 16.7 years). Self-reported ethnicities, categorized as per previous studies and as used within the NHS,[1] demonstrated the following in order of decreasing frequency: 15/47 Black (32%), 14/47 Asian (30%), 5/47 South East Asian (11%), 4/47 White (9%), 1/47 Mixed (2%) and in six patients ethnicity was not stated.

Skin phenotype

Detailed phenotypic descriptions are shown in supplementary table 1. In 63% of cases EDM was multifocal, and involvement of the periocular region (i.e. previously naevus of Ota) was frequently part of a multi-focal phenotype (**Figure 1**). Neck/shoulder involvement (i.e. previously naevus of Ito) was not specifically seen. Periocular involvement was part of the phenotype in 36% of cases,

predominantly located on the left side (71%) and the majority were female (88%). Nine patients presented with one large (>10 cm) well-defined lesion on the trunk (5) and limbs (4). Other cutaneous findings observed in decreasing order of frequency were: macular hyperpigmentation in the form of small café-au-lait macules, with fewer than four (6/47) or larger irregular macular hyperpigmentation (4/47), single hypopigmented macule (3/47), Blaschkolinear hypopigmentation (1/47), single congenital melanocytic naevus (4/47), epidermal naevus (1/47), smooth muscle hamartoma (1/47) and dermatofibrosarcoma protuberans (1/47).

Eye abnormalities

Thirty-four patients had an ophthalmological evaluation for which medical records or clinical information were available. Eighteen patients (38%) had scleral melanocytosis, one had abnormal choroidal pigmentation on fundoscopy, and another had borderline increased intraocular pressure. Peri-ocular skin involvement by EDM was significantly associated with ipsilateral scleral melanocytosis ($p<0.001$, two-tailed Fisher's exact), however congenital eye involvement was seen without peri-ocular EDM, and vice versa. Moreover, in the five patients with scleral melanocytosis without periocular EDM, both eyes were affected (**Supplementary table 1**).

Other abnormalities

Clinical symptoms or signs prompting concern for neurological involvement were the second commonest group. Two patients had other genetic diagnoses that could explain their neurological features: *SCN1A* related epilepsy in one and 22q11.2 deletion syndrome (DiGeorge syndrome) in another and were excluded from the following analyses. The remaining seven patients (16%)

showed a range of clinical symptoms or signs which prompted further evaluation: in one patient each, recurrent facial nerve palsy (patient 9), suspicion of coexisting capillary malformations which were later not confirmed (patient 16), headaches and concerns for attention and behavioural problems (patient 37), macrocephaly (patient 40) and speech and language delay in three. The first five of these had a brain MRI and two showed signs of an ischaemic infarct. The other three patients had normal intracranial findings (**Supplementary table 1**).

Genetic findings

Genetic investigations discovered new EDM-associated pathogenic mosaic variants in four patients, in one patient each (**Table 1 and Supplementary Figure 1**). The first was *HRAS* c.37G>C p.(Gly13Arg) at 21.73% VAF in patient 19 who presented with multiple congenital grey-blue macules on the upper back, shoulder and both arms, and less intensely blue lesions on the buttocks and lower limbs (**Figure 2**). The second was *ACTB* c.440G>C, p.(Arg147Pro) at 8% VAF in patient 41 who had a single left facial and periocular EDM and a separate small lesion suggestive of a smooth muscle hamartoma on the left mid-back (**Figure 3**). The third was *PIK3CA* c.1636C>A, p.(Gln546Lys) at 18% VAF in patient 26, who presented with multiple EDM on the whole back, buttocks and right arm, a Blaschkolinear pigmented keratinocytic epidermal naevus on the left chest and arm, and Blaschkolinear hypopigmentation, bilaterally on the chest (**Figure 4**). The last was *GNA11* c.547C>T p.(Arg183Cys) at 1% VAF in patient 13 with a large (>10 cm) dark, well-defined blue lesion on right lower back and buttock. He also showed congenital irregular café-au-lait macular pigmentation on the left chest, from which the *GNA11* variant was not detected by

sensitive methods (**Figure 5**). In addition, we identified previously described mosaic *GNAQ* variants in four further cases, all affecting codon 183 (**Figure 6**). In all cases tested the mosaic variant was absent in the blood using sensitive methods.

Discussion

The main objective of our study was to deepen our understanding of EDM by combining deep phenotyping with genetic investigations in a large paediatric cohort. This approach allowed us to gain valuable insights into several aspects of the disease.

Interpretation of the demographic and phenotypic data in our cohort was not straightforward. There was a significant preponderance of females with facial EDM over males, but no significant difference for other body locations. It is therefore possible that this is a reflection of societal concerns regarding gender differences in cosmetic appearance rather than a true difference in location between the sexes. Along similar lines, periocular involvement was seen in 36% of patients, and was often the lesion highlighted in referral letters despite frequently being part of a multiple EDM phenotype. In contrast, only 19% of patients presented with a large lesion on the trunk or limbs. Periocular involvement may therefore be more likely to prompt a referral for specialist evaluation. The self-reported ethnicity profile in our cohort is in agreement with previous studies, suggesting that EDM is commoner in darker skinned populations[1]. However, now that we have more mosaic genotypic evidence we consider it is possible that EDM is simply more easily discernible on darker skin tones (see **Figure 6** for examples of *GNAQ* mosaicism on different background skin tones). In support of this idea, we know already that the colour of

congenital melanocytic naevi, another mosaic disorder, is linearly associated with an individual's background skin tone and germline pigmentary genotype[22]. While congenital melanocytic naevi are readily visible on all skin tones, EDM is a much subtler phenotype in general, and therefore could be missed if the depth of pigmentation is less pronounced. Until more is known clinicians should therefore continue to be alert to the possibility of EDM in all locations and in all skin tones.

Eye involvement was the commonest extracutaneous finding in this cohort, and as it was not restricted to those with periocular skin involvement, we would recommend that all patients with a diagnosis of EDM be reviewed at least once by an ophthalmologist. Ophthalmologists are likely to be best placed to differentiate common mild scleral pigmentation related to background ancestral skin phenotype from oculodermal melanocytosis. The interesting finding that those with eye involvement without periocular skin involvement had bilateral eye involvement potentially suggests a different disease mechanism for that specific phenotype. Those patients currently have no causative genotype. We consider that the terms "naevus of Ota" and "naevus of Ito" are probably now redundant, as both are recognized to be part of the EDM disease spectrum, and as they often occur in the context of multiple lesions which share a common underlying genetic cause.

Assessment of CNS involvement in this study was not done systematically. However, two patients of five scanned (one with a large EDM lesion on the leg and another with facial EDM) showed signs of CNS ischemic infarcts, without other cause. Their genotype remains unknown and the

potential relationship of these infarcts to the EDM is currently unclear. It is however already known that the spectrum of *GNAQ* and *GNA11* mosaicism includes both pigmentary and vascular abnormalities in skin, eye and brain, and that these are clonal diseases[23,24]. Leptomeningeal melanocytic disease in the setting of EDM has been previously reported[10,24]. One patient with *GNA11*-phakomatosis pigmentovascularis (PPV) with dermal melanocytosis showed a severe neurological phenotype with recurrent status epilepticus for which he underwent left posterior quadrantectomy, revealing both increased leptomeningeal melanocytes and abnormal vasculature on histopathological examination[24]. Therefore it is conceivable that EDM on the skin could be associated with vascular abnormalities in the CNS which could give rise to stroke, although lack of underlying vascular abnormality detectable on MRI may argue against this. Going forwards within our service we will be undertaking MRI screening of EDM cases to delineate the incidence and nature of congenital CNS disease. We would recommend in the interim that CNS symptoms and signs are sought clinically, and any potential abnormalities investigated.

We identified the underlying genetic cause of EDM in 29% of those who underwent genetic investigation. Somatic activating mutations in *GNAQ* were the commonest cause, present in 16% of our cohort, however overall numbers in this rare disease are still very low. Mosaic pathogenic variants in *GNA11, ACTB*, *HRAS* and *PIK3CA* genes were identified in one patient each, representing new mosaic causes of EDM. Each of the variants is already known to be pathogenic, and already established to be the cause of other mosaic disorders affecting the skin[23,25-27], but not to cause EDM. The specific *GNA11* variant found here is already established as an alternative

genotypic cause of other *GNAQ*-mosaic phenotypes Sturge-Weber syndrome and PPV[14,15]. In addition, in the patient with EDM and a Blaschkolinear epidermal naevus we detected the same variant in both lesions, demonstrating clonality of the phenotype and therefore causality. From a technical perspective, diagnosis was within a regional diagnostic genetics service and results were validated by two different methods.

We are interested to see going forward whether the clinical risks stratify by genotype. Oncogenic *GNAQ* and *GNA11* variants are more commonly associated with sporadic uveal melanoma than the other genes identified[28]. EDM appears to be more commonly associated with variants affecting codons 183 than codon 209 of either protein, whereas sporadic melanoma is more commonly associated with variants affecting codon 209 than 183[21,29]. Although the exact molecular events driving progression from EDM to melanoma are not fully understood[30-32], in the documented cases of patients with EDM developing melanoma which have included genetic analysis, *GNAQ* variants are common events[19,20,30,31]. It is therefore likely that *GNAQ* and *GNA11* variants are centrally involved in melanoma development in patients with EDM. We will however need adequate numbers and follow up before any conclusions regarding melanoma risk and genotype can be made, and all EDM patients should be made aware of potential risk of melanoma. There is however potentially a genotypic difference with potential for germline transmission to offspring, as the *HRAS* c.37G>C p.(Gly13Arg) variant has been described as a rare cause of Costello and Noonan syndromes[33], whereas all other variants have not so far been described in the germline. Genetic counselling should therefore be patient and variant specific as already recommended for mosaic disorders[34].

The presence of other birthmarks may be helpful in clinical diagnosis. In addition to *PIK3CA*- EDM with epidermal naevus we also observed a probable smooth muscle hamartoma in *ACTB*-EDM. Although the *ACTB* variant was not detected in the smooth muscle hamartoma we propose to rebiopsy when the phenotype may be more developed. Areas of café-au-lait macular hyperpigmentation without a clear developmental pattern have already been described as a clinical feature of patients with EDM[15,35], however there is no previous sequencing data from these lesions. The absence of a pathogenic variant in one such lesion here (**Figure 5b**) suggests these may be have a different origin, or that the variant was below detection limits.

Patients are sometimes referred for consideration of treatment for cosmetic reasons. Although EDM does not self-resolve as rapidly as transient blue spots, we have anecdotally observed substantial reduction in pigmentation over the first decade, and we recommend colour-standardised serial photography to assess evolution. Laser therapy has also been used with variable success for EDM[36]. Genotypic investigation should herald options for future novel therapies, in the context of recent progress targeting mosaic disorder variants including *GNAQ*, *GNA11* and *NRAS*[37,38].

Taken altogether, these findings emphasize the need to diagnose and investigate EDM, which may easily be overlooked because of the clinical overlap with common transient blue spots. The discovery of multiple mosaic causes in EDM contributes to a better understanding of the

molecular landscape. Ongoing research is needed to clarify the genotype-outcome relationships and further direct clinical management.

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Figure Legends

Figure 1. Cutaneous phenotype of EDM

(a) Left sided facial EDM, with forehead, periocular and cheek involvement previously termed naevus of Ota. **(b)** Multiple EDM on the back in a patient with facial EDM. **(c)** Multiple EDM on the back with deeper pigmented lesions on the upper back and flank in a patient without facial EDM.

Figure 2. Clinical appearance of *HRAS*-related EDM

(a-c) Multiple congenital grey-blue macules on upper back, shoulder and both arms, with less intensely blue lesions of the same type on buttocks and lower limbs. **(c)** Two café-au-lait macules are shown on the right buttock and left thigh. The circled area indicates the site from which the skin biopsy was taken.

Figure 3. Clinical appearance of *ACTB*-related EDM

(a) Left sided facial EDM with forehead, periocular and scalp involvement in a patient with scleral melanocytosis in both eyes. The circled area indicates the site from which the skin biopsy was

taken. **(b)** Multiple less intensely blue lesions of the same type on the whole back and small separate lesion suggestive of a smooth muscle hamartoma on the left mid-back. **(c)** Histological findings of EDM from the scalp biopsy, showing numerous spindle-shaped and dendritic melanocytes and melanophages within the upper and mid-dermis (Hematoxylin and eosin stain, 100µm). **(c)** Histological findings of smooth muscle hamartoma on the left mid-back, showing smooth muscle bundles dispersed throughout the dermis (Hematoxylin and eosin stain, 100µm). Noted in both, hyperpigmentation of the basal layer related to patients' dark skin type.

Figure 4. Clinical appearance of *PIK3CA*-related EDM

(a-b) Multiple EDM on the whole back, buttocks and right arm. **(c)** Blaschkolinear pigmented keratinocytic epidermal naevus on the left arm. **(d)** Blaschkolinear hypopigmentation on the chest, with bilateral involvement (have more pics, linear right and going down the centre but then also on the left side – more linear 'coma' shaped. Blaschkoloinear epidermal naevus is also shown on the left chest.

Figure 5. Clinical appearance of *GNA11*-related EDM

(a) Single large EDM lesion on the right lower back and buttock with less intensely pigmented lesions of the same type on the buttocks. The skin biopsy was taken from the more deeply pigmented area, as indicated by the presence of biopsy scars. **(b)** Irregular café-au-lait macular pigmentation on the chest.

Figure 6. Clinical appearance of *GNAQ*-related EDM

(a) Multiple EDM on the buttocks, abdomen, legs and feet (not shown). **(b)** Single large EDM lesion on the left leg with less intensely pigmented lesions of the same type on the lower back and buttocks **(c-d)** Single large EDM lesion on the right torso, also showing mild eczema**.** All three caused by a mosaic *GNAQ* variant affecting codon 183. The circled area indicates the site from which the skin biopsy was taken.

Supplementary Figure 1. New genotypes associated with EDM: sequencing results demonstrating mosaic variants in genes *GNA11*, *HRAS*, *ACTB* and *PIK3CA*.

Next generation sequencing (NGS) data from affected skin samples, viewed in the Integrated Genomic Viewer (IGV) demonstrating **(a)** a mosaic missense variant in *GNA11* NM_002067.5:c.547C>T p.(Arg183Cys) at 1% VAF (16/1441 reads); **(b)** a mosaic missense variant in *HRAS* NM_005343.4:c.37G>C p.(Gly13Arg) at 21% VAF (611/2866 reads); **(c)** a mosaic missense variant in *ACTB* c.440G>C, p.(Arg147Pro) at 8% VAF; and **(d)** a mosaic missense variant in *PIK3CA* NM_006218.4: c.1636C>A, p.(Gln546Lys) at 18% VAF.

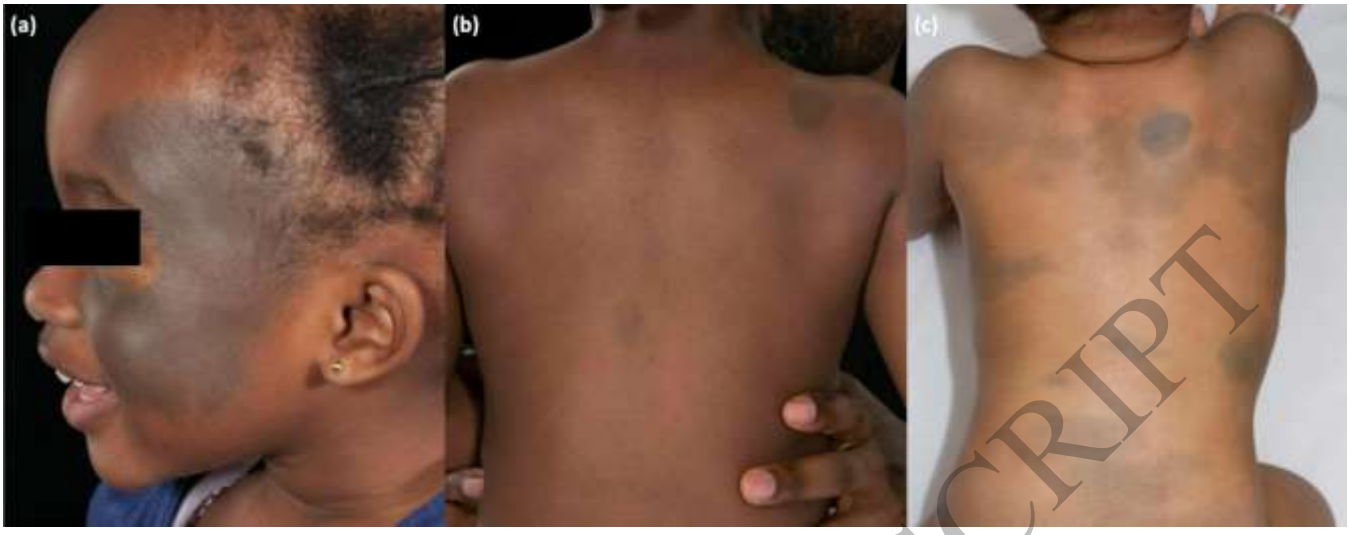
1 **Table 1 -** Phenotype and genotype of all patients with a mosaic genotype established in skin.

Patient No.	Sex	Age at first presentation, years	Self-reported Ethnicity	Cutaneous phenotype	Ophthalmological phenotype	Other medical history	Ger DN
2*	F	1.5	Asian	Multiple congenital blue-grey macules, whole back and arm	-	-	*GNA* p.(A arm
3*	M	0.2	NS	One large (>20 cm) dark, well-defined lesion, flank and less intensely blue lesions of the same type, lower back and buttocks	-	-	*GNA* p.(G flar
13	M	0.4	White	One large (>10 cm) dark, well-defined lesion, right lower back and buttock and less intensely blue lesions of the same type, whole back, buttocks and shoulders. Irregular café-au-lait macular pigmentation, chest and leg	-	Speech and language delay	*GNA* p.(A bac
14	M	0.4	NS	Multiple congenital dark blue-grey macules, right abdomen, buttocks, right leg and foot	-	-	*GNA* p.(A bac
15	F	0.5	White	One large (>10 cm) blue-grey lesion, right torso	-	-	*GNA* p.(A
19	F	0.2	Black	Multiple congenital grey-blue macules, upper back, shoulder and both arms, with less intensely blue lesions of the same type, buttocks and lower limbs. Three areas of irregular café au-lait macular pigmentation (ranging from 1 to 3 cm), chest, buttock and leg	-	-	*HRA* p.(G bac
26	M	0.3	Black	Multiple congenital grey-blue macules, whole back,	-	-	*PIK3* c.16

				buttocks and right arm. Blaschkolinear pigmented epidermal naevus, left chest and arm. Blaschkolinear hypopigmentation, bilateral chest			VAF 14%
27	F	0.9	Other	One large (>10 cm) blue-grey lesion, left leg and less intensely blue lesions of the same type, lower back and buttocks	-	-	*GNA* p.(A
31	F	2.9	Black	Right sided facial EDM, with periocular involvement	Scleral pigmentation, choroidal pigmentation, right eye	Benign tonic upgaze in infancy (resolved), speech delay, concerns about attention	*GNA* p.(A fac
41	F	3.0	Black	Left sided facial EDM, with forehead, periocular and scalp involvement and smooth muscle hamartoma, right mid-back	Scleral pigmentation, both eyes	Speech and language delay (resolved)	*ACT* p.(A sca

*indicates a patient previously reported in[14]

Abbreviations: VAF, variant allele frequency; F, female; M, male; NS, not stated



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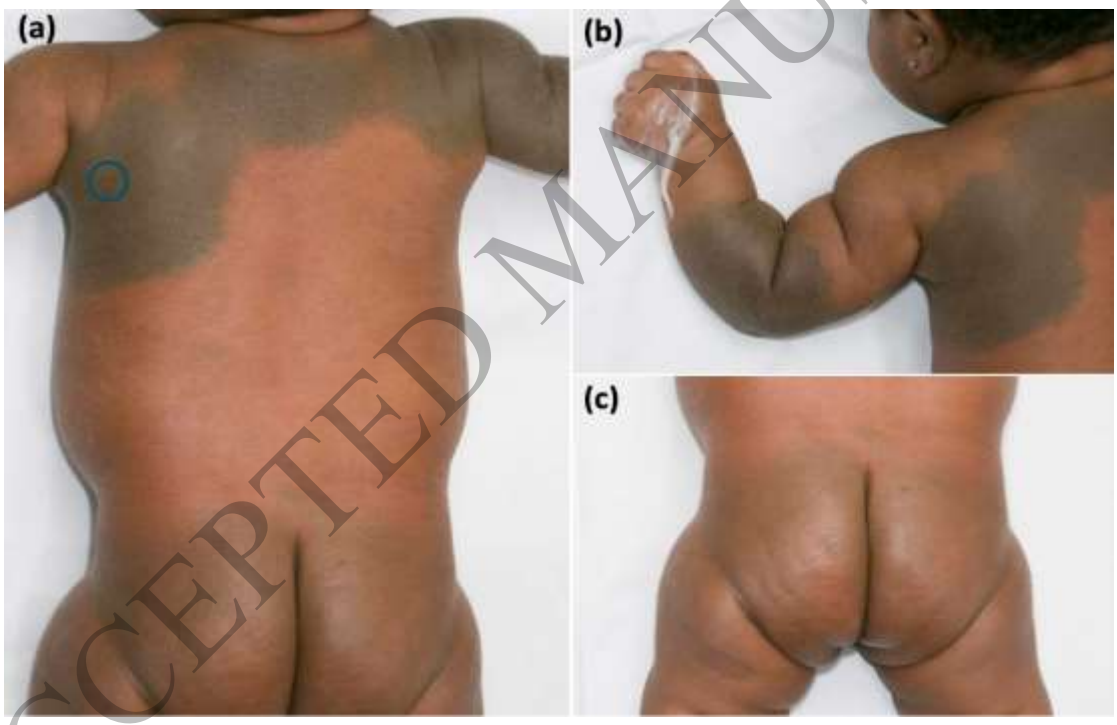

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Figure 3
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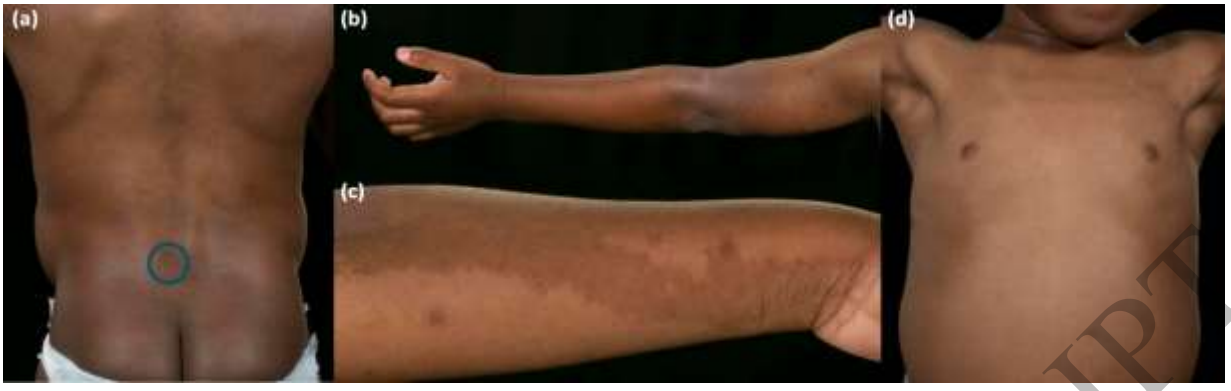

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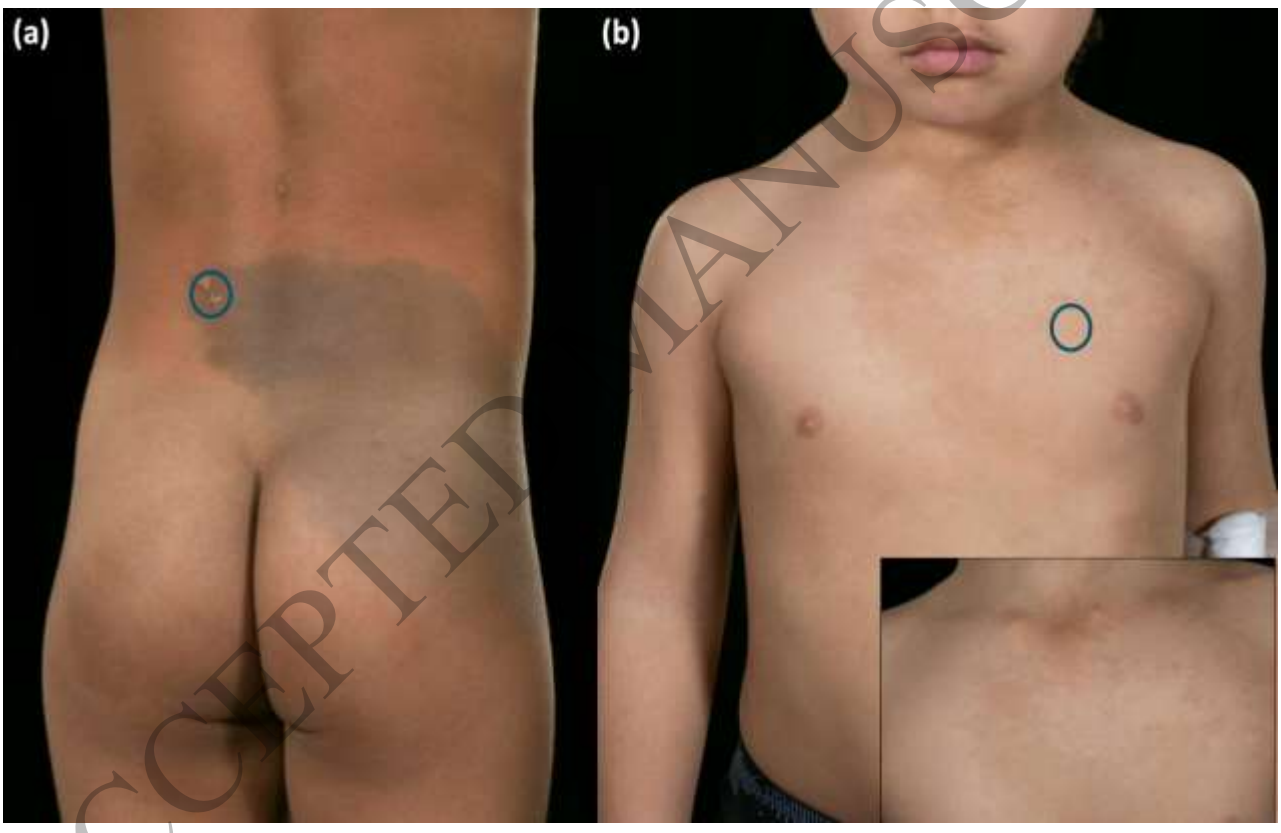

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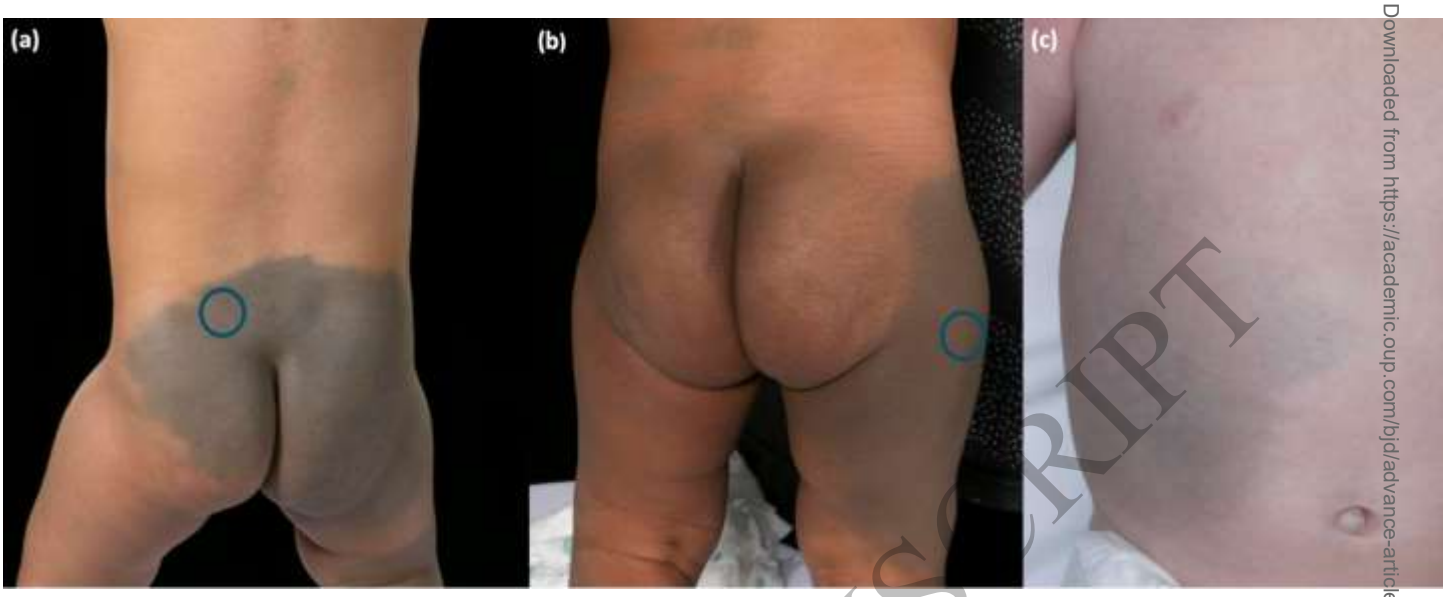

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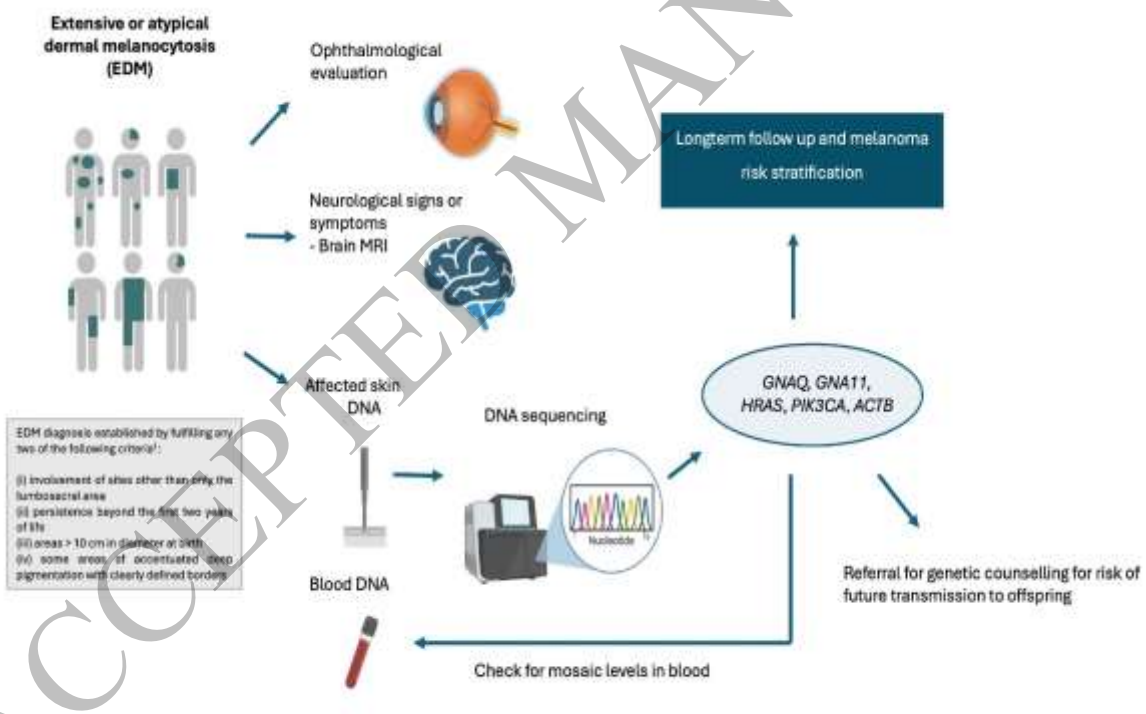

Graphical Abstract
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