The Distinctive Genomic Landscape of Giant Congenital Melanocytic Nevi

TO THE EDITOR

Congenital melanocytic nevi (CMN) are heterogeneous in their clinical appearance and implications and range in size from small to large and/or giant. Large—giant CMN (LGCMN) have an increased risk of malignant transformation, and the most severe complication manifests as melanoma of the CNS. Mutations in the oncogene NRAS are the most frequently observed; however, this postzygotic mutation is not present in all subtypes of CMN. In a study of LGCMN, Martins da Silva et al. (2019) performed RNA sequencing and multiregional sequencing (median coverage approximately ×19,000) using a targeted panel of genes (see Supplemental Materials) and discovered that ~60% harbored an NRAS mutation, but other rare events such as gene fusions were identified. To date, there have been a limited number of CMN assessed using whole-genome (Colebatch et al., 2019) or whole-exome sequencing (Charbel et al., 2014; Lim et al., 2020; Melamed et al., 2017), with NRAS mutations reported at a high frequency in all studies (range, 80–100%). In consideration of our previous study, whereby ~40% of LGCMN have no known driver mutation present, we sought to genomics characterizing lesions from patients with LGCMN with known NRAS mutation status (mutant and wild type [WT]) to discover other potential somatic driver mutations and expand our knowledge of the mutational spectrum present in LGCMN.

After institutional approval of experiments and written informed patient consent (see Supplemental Materials), we performed whole-exome sequencing of eight affected skin biopsies (lesional) from five patients with giant CMN (age range, 4–58 years) with matching unaffected skin (not available in one patient) along with germline DNA (Figure 1 and Supplementary Materials and Supplementary Table S1). In all the patients, we analyzed at least one biopsy of the largest CMN lesion, including a second biopsy of this lesion from two patients and an additional biopsy of a satellite lesion in another patient (Table 1). To ensure that high coverage was achieved across the exome region, sequencing was performed at a mean depth of ×135–204 for all CMN and matching unaffected skin and ×76–93 for germline DNA (Supplementary Table S2). The sequencing data were analyzed as previously described (Stark et al., 2020, 2018) and described briefly in the Supplemental Materials.

Somatic single-nucleotide variants were identified in all the lesional samples from the exome pull-down region with ranges from 35 to 66 mutations (median, 49) or 0 to 1 mutations per megabase (Supplementary Table S3). The total number of insertion—deletion mutations was 36–185 (median, 101), which was approximately two times higher than that of the single-nucleotide variants (Supplementary Table S3). This high proportion of insertion—deletion mutations (median, 69%) is consistent with our studies of acquired nevi located on the body sites with minimal sun-exposure (Stark et al., 2020).

We confirmed the presence of the common NRASQ61R mutation in all previously known NRAS mutated lesions (Table 1 and Supplementary Table S4). Along with the common NRAS driver mutation, other mutations were present at a high mutation frequency (Supplementary Tables S5 and S6). For example, in paired samples from patient 5, a nonsynonymous mutation in Keratin 81 (KRT81; NM_002281:exon2:c.C415A:p.Q139K) occurred at an equally high mutation frequency.

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frequency, indicating that it co-occurred with the NRAS<sup>p.Q61R</sup> mutation. However, the NRAS<sup>p.Q61R</sup> mutation in biopsies of patient 5 was not at an equally high frequency, which is consistent with our previous report (Martins da Silva et al., 2019) (Supplementary Table S4). KRT81 has recently been found to be associated with an invasive phenotype in breast cancer cell lines (Nanashima et al., 2017); however, the p.Q139K is not predicted to be deleterious (Supplementary Table S5). It is interesting to speculate whether KRT81 is playing a more dominant role in the CMN development in this patient.

Regarding the two patients with WT NRAS (patients 2 and 3), by determining the highest protein-altering mutation frequency, we identified potential novel driver mutations (Supplementary Tables S4 and S5). In patient 3, we found the p.T16M mutation (NM_013390: exon24: c.3956-2->TT) in the cell migration gene (LFNG) (Supplementary Tables S4 and S5). This mutation was found to have the highest mutation frequency in this sample (15%). Unfortunately, the presence of the TMEM2 mutation could not be assessed in any additional biopsy (Figure 1); however, copy number loss (loss of heterozygosity) was observed in D16-0090 (Supplementary Table S8).

LFNG is a member of the NOTCH-signaling pathway and plays a crucial role in somitogenesis during embryonic development (Sparrow et al., 2006). Interestingly, a loss of LFNG was recently found to be associated with promoting melanoma metastasis (Del Castillo Velasco-Herrera et al., 2018; Raimo et al., 2016), possibly acting through the NOTCH/PTEN/Akt pathway (Raimo et al., 2016). Because nevi are primarily driven by MAPK pathway activation through BRAF/NRAS mutations, conceivably, the LFNG mutation points to an alternative pathway to nevogenesis.

In the other patient with WT NRAS (D16-0064), we found a splice-site mutation (NM_002304:exon24: c.3956-2->TT) in the cell migration gene (TMEM2) (Supplementary Table S5). This mutation was recently found to be associated with promoting melanoma metastasis (Schinzel et al., 2019). Because CMN are largely driven by MAPK signaling, the splice-site mutation in TMEM2 would be a strong candidate. The role of these potential driver mutations in LFNG and TMEM2 in CMN development is yet unknown and warrants further investigation both at the functional level and in an expanded cohort of NRAS WT CMN.

Next, we performed a somatic mutation signature analysis using an updated mutation signature framework (Alexandrov et al., 2020). In a previous study of acquired melanocytic nevi (Stark et al., 2020), the UV-related single-base substitution (SBS) 7 signature was a dominant feature in predominantly sun-exposed nevi and was absent in the nevi with limited or no sun-exposure. In this study, there were no classical UVR signature mutations (SBS7) detected. The lack of SBS7 signatures reflects the congenital origin of these lesions, which are not induced by UVR exposure. Instead of UVR-related SBS7 signatures, the signatures associated with defects in mismatch repair (MMR) (SBS15, SBS21, SBS44) were the most frequently observed (combined total, 6 of 8 or 75%; Table 1 and Supplementary Table S7). MMR-related signatures are also common in acquired nevi and are often present in the absence of SBS7 (Stark et al., 2020). In the satellite CMN of patient 3 (D16-0091; Figure 1), no detectable MMR signatures were found, whereas the larger lesion had MMR present, thus highlighting the heterogeneous nature of the giant CMN (Figure 1). The precise cause of the MMR signatures is unknown because there were no corresponding somatic mutations present, but patients carried rare germline variants in MMR pathway genes such as MSH2, MSH3, PM11, and PM2 (data not shown). In further supportive evidence, signatures relating to MMR have been previously detected in CMN (Colebatch et al., 2019), which suggests that the MMR pathway contributes to congenital nevogenesis.
The next most frequent signatures were SBS39 (63%) and the ubiquitous SBS5 (50%; both have unknown etiology [Alexandrov et al., 2020]) and SBS32 (50%; Table 1 and Supplementary Table S6). SBS32 is commonly found in squamous cell carcinomas derived from patients with transplantation that have been treated with the immune-suppressive drug azathioprine (Inman et al., 2018). However, because SBS32 has also been detected in acquired melanocytic nevi with no reported azathioprine therapy (Stark et al., 2020), it can be suggested that this signature may also result from an unknown process that remains to be elucidated.

Finally, we also determined the degree of copy number aberrations (CNAs) in the samples with matching normal skin available (Supplementary Tables S8 and S9). Atypical nodular proliferations, arising from congenital nevi, are known to contain CNAs (Bastian et al., 2002). The total CNAs ranged from 6 to 649 and were mostly in small regions of loss and/or gain (mean, 530 kilobases), which is in contrast to acquired nevi (mean, 8.3 megabases) (Stark et al., 2018). Heterogeneity of the CMN was also apparent in paired patient samples, with broad ranges of CNAs observed (Supplementary Tables S8 and S9). Copy number loss was observed in chromosomal regions encompassing known oncogenes (BRAF, SETDB1, MDM2, RAC1, PARP1, and DDX43) as well as tumor suppressor genes (TP53 and PPP6C) (Supplementary Tables S8 and S9). We have previously noted balanced CNA events in acquired melanocytic nevi (Stark et al., 2020, 2018), which again was the norm in this collection of CMN. Conversely, there were no imbalanced CNA events, which are common in melanoma.

In summary, we have discovered potential novel driver mutations in WT NRAS giant CMN, representing alternative mechanisms for the development of these lesions. In addition, we found that giant CMN show a distinctive molecular profile comprising mutational signatures associated with MMR genes and short CNAs. Further studies are necessary to explore the role of LFNG, TMEM2, and MMR genes in giant CMN development.

### Data availability statement

Alignment files (.bam) generated in this study have been submitted to the European Genome-phenome Archive (https://ega-archive.org/) under accession number EGAS00001004541.

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### CONFLICT OF INTEREST

HPS is a shareholder of MoleMap New Zealand Limited and e-derm-consult Gesellschaft mit beschränkter Haftung and undertakes regular tele-dermatological reporting for both companies. HPS is a medical consultant for Canfield Scientific, MetaOptima Technology, and Revenio Research Oy and a medical advisor for First Derm. The remaining authors state no conflicts of interest.

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### Table 1. Mutational Spectrum Detected in Lesions from Patients with Giant CMN

<table>
<thead>
<tr>
<th>Patient</th>
<th>CMN Size (cm)</th>
<th>Biopsy ID</th>
<th>Type of Lesion</th>
<th>Body Site</th>
<th>Driver Mutation (MAF)</th>
<th>Total</th>
<th>SNVs</th>
<th>INDELS</th>
<th>SBS MMR Signature, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Giant (&gt;60 cm)</td>
<td>D16-0062</td>
<td>CMN</td>
<td>Trunk—right lower back</td>
<td>NRAS p.Q61R (28%)</td>
<td>35</td>
<td>93</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2 Giant (40–60 cm)</td>
<td>D16-0064</td>
<td>CMN</td>
<td>Scalp</td>
<td>TMEM2 c.3956-2&gt;TT (15%)</td>
<td>43</td>
<td>46</td>
<td>15</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3 Giant (&gt;60 cm)</td>
<td>D16-0090</td>
<td>CMN</td>
<td>Trunk—right scapula</td>
<td>LFNG p.T16M (30%)</td>
<td>44</td>
<td>36</td>
<td>9</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4 Giant (&gt;60 cm)</td>
<td>D16-0091</td>
<td>Satellite Trunk—inferior abdomen</td>
<td>LFNG p.T16M (23%)</td>
<td>66</td>
<td>185</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Giant (&gt;60 cm)</td>
<td>D16-0105</td>
<td>CMN</td>
<td>Trunk—left scapula</td>
<td>NRAS p.Q61R (23%)</td>
<td>51</td>
<td>114</td>
<td>18</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4 Giant (&gt;60 cm)</td>
<td>D16-0115</td>
<td>CMN</td>
<td>Trunk—right hip</td>
<td>NRAS p.Q61R (29%)</td>
<td>52</td>
<td>129</td>
<td>12</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5 Giant (&gt;60 cm)</td>
<td>D16-0110</td>
<td>CMN</td>
<td>Trunk—right scapula</td>
<td>NRAS p.Q61R (1%)</td>
<td>51</td>
<td>108</td>
<td>11</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1 Giant (&gt;60 cm)</td>
<td>D16-0111</td>
<td>CMN</td>
<td>Genital—right upper lip</td>
<td>NRAS p.Q61R (22%)</td>
<td>47</td>
<td>82</td>
<td>10</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CMN, congenital melanocytic nevi; ID, identification document; INDEL, insertion–deletion; MAF, mutant allele frequency; MMR, mismatch repair; SBS, single-base substitution signature; SNV, single-nucleotide variant.

Summary of known and potential novel driver mutations in CMN biopsies and their associated MAF (%). The total number of SNVs were approximately two-fold less than the total number of small INDELS. SBS mutational signatures (proportion present, %) were commonly associated with defects in MMR. All somatic SNVs were detected in the coding and noncoding region, including splice sites and dinucleotide variants.
Can People Correctly Assess their Future Risk of Melanoma?

TO THE EDITOR

Prospectively identifying people at increased risk for melanoma is a key prerequisite to designing interventions aiming to detect melanoma early when treatment has a high probability of being curative. Whereas population-based screening for melanoma is not recommended because there is no evidence that it decreases mortality (Wernli et al., 2016), medical authorities in the United States, Australia, and elsewhere advocate targeted screening of people at a high risk. What is unknown, however, is whether people can accurately assess their own personal risk of developing melanoma. Self assessments in European populations have been shown to be only moderately accurate in identifying people at a high risk, correlating poorly with dermatologists’ evaluations on melanoma risk factors (Carli et al., 2003; Harbauer et al., 2003; Richtig et al., 2008). Moreover, there is evidence to suggest that people are not confident in assessing their future risk of melanoma and can be reluctant to seek advice from their treating doctors (Eiser et al., 2000).

**Abbreviations:** CI, confidence interval; RR, risk ratio

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**REFERENCES**


**Materials and Methods**

**Patients and Nevis Specimens**

Patients diagnosed with large or giant congenital melanocytic nevi (CMN) who attended the Hospital Clinic of Barcelona, Spain, between 2013 and 2015 were included. Skin biopsy samples were taken from different phenotypic areas as indicated in Figure 1. Clinical information (sex, age at the time of biopsy, previous history of melanoma or neurocutaneous melanosis) was collected in all the patients. Patients were classified according to the Krengel classification (Krengel et al., 2013), and those with giant CMN were classified by the B6 classification (Martins da Silva et al., 2017). All CMN were classified as classic CMN. Written informed consent was obtained from patients, or from the patients’ parents and/or guardians, and the study was approved by the medical ethics committee of the Hospital Clinic of Barcelona and complied with the Declaration of Helsinki Principles. Patients, or the patients’ parent and/or guardian, consented for the publication of their images where presented in this study (except patient 4).

DNA was extracted from fresh tissue using the QiAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. DNA was quantified using a Qubit dsDNA BR Assay Kit (Invitrogen, Waltham, MA).

**Exome Capture and Illumina Sequencing**

Exome capture was performed using the Agilent SureSelect Target Enrichment System, Human All Exon V5+UTRs kit (Agilent Technologies, Santa Clara, CA), according to the manufacturer’s instructions. Sequencing of germline DNA from the five study participants along with normal skin (4 of the 5 participants) and congenital nevi biopsies (seven in total, with two paired specimens) was carried out on an Illumina NovaSeq6000 platform (Illumina, CA) with paired-end 100 basepair reads following Illumina-provided protocol by a fee-for-service provider (Macrogen, Seoul, Republic of Korea). Coverage statistics for the capture regions were generated with Genome Analysis Toolkit, version 3.1-1 (McKenna et al., 2010).

**Read Mapping, Data Preprocessing, and Somatic Variant Calling**

The analysis pipeline has been previously described (Stark et al., 2018).

**Somatic Variant Calling and Filtering**

Somatic variants present in the CMN and normal skin biopsies were determined by filtering out all the variants that were present in the matching blood-derived germline DNA, followed by all variants that were present in the European population in the 1,000 genomes project (version 1000g2014oct_eur) and Exome Aggregation Consortium Non-Finnish European databases. Variants present in Single Nucleotide Polymorphism Database (version 138) were not used as a filter owing to the presence of somatic mutations (e.g., BRAN600E). Accordingly, a proportion of the variants presented in Supplementary Table S6 may be polymorphisms. Next, to generate a list of somatic mutations that were present only in the CMN biopsies, we pooled all the somatic mutations that were found in the four normal skin biopsies and removed these from the CMN data. To minimize the chance of a rare variant or commonly occurring somatic variant (potentially sequencing artifact), any variant present in a pool of 30 normal skin samples (Stark et al., 2018) were also removed from the CMN samples, resulting in a list of somatic variants present only in the CMN. Next, all the somatic variants present in the nevi were filtered further according to the criteria previously described (Stark et al., 2018) and are presented in Supplementary Table S6.

**Detection of Known NRAS Mutations**

The common NRAS Q61R somatic mutation was known to be present in the CMN samples D16-0062, D16-0105, D16-0115, D16-0110, and D16-0111 and confirmed to be absent (NRAS wild type) in D16-0064, D16-0090, and D16-0091. The NRAS wild-type samples were also known to be the wild type for somatic mutations in BRAF, AKT1, EGRF, GNAS, ALK, ERBB2, KIT, APC, FBXW7, KRAS, STK11, PDGFR, TP53, PIK3CA, BRAF, GFR2, MAP2K1, Pten, CDH1, FOX2L1, MET, CTNNBN1, GNAQ, MSH6, SRC, and SMAD4 (Martins da Silva et al., 2019). We confirmed the NRAS Q61R mutation in 5 of the 5 CMN; however, because D16-0110 was known to have a low mutant frequency (Martins da Silva et al., 2019), it was not present in the stringently filtered list of mutations (Supplementary Table S6) and was only detectable in the Binary Alignment Map file (visualized using the Integrative Genome Viewer [Broad Institute, Cambridge, CA]) (Supplementary Table S4).

**Mutation Signature Analysis**

Filtered somatic single-nucleotide variants present in the CMN were imported into the deconstructSigs (Rosenthal et al., 2016) package using R 3.4.0 for Windows and were analyzed within the CNVkit (Talevich et al., 2016) package (https://github.com/etal/cnvkit) and run using Python 2.7. Only the CMN that had matching normal skin were able to be analyzed for copy number aberrations. Briefly, the CMN (D16-0062, D16-0064, D16-0090, D16-0091, D16-0105, and D16-0115) and matching normal skin Binary Alignment Map files, with duplicates marked and sorted (see methods above) were analyzed within the CNVkit according to the standard methods (Talevich et al., 2016). A segmentation file (Supplementary Table S8) was compiled from all the lesions. Genes involved in regions of gain (copy number of >3) and loss (copy number of <1), summarized in Supplementary Table S9, were those commonly mutated in melanoma (Cancer Genome Atlas Network, 2015).

**Supplementary References**


