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Activating cysteinyl leukotriene receptor 2 (*CYSLTR2*) mutations in blue nevi

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Abstract

Blue nevi are common melanocytic tumors arising in the dermal layer of the skin. Similar to uveal melanomas, blue nevi frequently harbor *GNAQ* and *GNA11* mutations. Recently, recurrent *CYSLTR2* and *PLCB4* mutations were identified in uveal melanomas not harboring *GNAQ* or *GNA11* mutations. All four genes (*GNAQ, GNA11, CYSLTR2*, and *PLCB4*) code for proteins involved in the same signaling pathway, which is activated by mutations in these genes. Given the related functional consequences of these mutations and the known genetic similarities between uveal melanoma and blue nevi, we analyzed a cohort of blue nevi to investigate whether

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Disclosure/conflict of interest

All other authors have nothing to declare.

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CYSLTR2 and *PLCB4* mutations occur in tumors lacking *GNAQ* or *GNA11* mutations (as in uveal melanoma). A targeted next-generation sequencing assay covering known activating mutations in *GNAQ, GNA11, CYSLTR2, PLCB4, KIT, NRAS*, and *BRAF* was applied to 103 blue nevi. As previously reported, most blue nevi were found to harbor activating mutations in *GNAQ* (59%, $n = 61$), followed by less frequent mutations in *GNA11* (16%, $n = 17$). Additionally, one *BRAF* (1%) and three *NRAS* (3%) mutations were detected. In three tumors (3%) harboring none of the aforementioned gene alterations, *CYSLTR2* mutations were identified. All three *CYSLTR2* mutations were the same c.386T > A, L129Q mutation previously identified in uveal melanoma that has been shown to lead to increased receptor activation and signaling. In summary, our study identifies *CYSLTR2* L129Q alterations as a previously unrecognized activating mutation in blue nevi, occuring in a mutually exclusive fashion with known *GNAQ* and *GNA11* mutations. Similar to *GNAQ* and *GNA11* mutations, *CYSLTR2* mutations, when present, are likely defining pathogenetic events in blue nevi.

Blue nevi are relatively common, usually dermal, melanocytic proliferations of the skin.[1] They are pigmented tumors, which appear blue when viewed clinically, due to their location beneath the skin surface, a physical phenomenon based on the Tyndall effect.[2] The most frequent subtype are so-called common blue nevi, or Jadasohn-Tièche blue nevi, based on their original description.[3] A range of other subtypes have been described,[1,2,4] including cellular, epithelioid, sclerotic, and plaque-type blue nevi. Congenital dermal melanocytosis, which include Mongolian spots, nevi of Ota, and nevi of Ito[4] are also included in the blue nevus group.

Most blue nevi are benign and rarely transform into malignant tumors. However, there are tumors designated atypical cellular blue nevi, the biological potential of which is difficult to determine from their clinicopathologic features. Additionally, so-called malignant blue nevi are clearly malignant melanomas and should be treated accordingly.[5–8]

Blue nevi show a distinct genetic profile from that of epidermal-derived common acquired nevi; the majority of the latter (80–90%) harbor activating *BRAF* V600E mutations,[9] which are not commonly found in blue nevi. Blue nevi harbor highly recurrent activating *GNAQ* and *GNA11* mutations, which are also found in uveal melanoma.[10,11] The two activating mutations reported recurrently in blue nevi to date are *GNAQ* mutations (~55%) and *GNA11* mutations (~7%).[11] A number of studies have further demonstrated mutations or protein loss of the tumor suppressor BAP1 occurring in malignant blue nevi.[5,12,13]

In all studies to date, there remain tumors (eg, 35–40% of blue nevi) lacking *GNAQ* or *GNA11* mutations, implying that they harbor other unrecognized activating oncogene mutations.[5,10,11] All known gene alterations reported in blue nevus variants to date (*GNAQ, GNA11*, and *BAP1*) are also frequently found present in uveal melanomas.[10,11,14–16] Recently, two studies demonstrated rarer mutations in *PLCB4*[17] and *CYSLTR2*[18] occurring in uveal melanomas lacking *GNAQ* or *GNA11* mutations. These four mutations (*GNA11, GNAQ, PLCB4*, and *CYSLTR2*) affect different proteins, and although the functional consequences of the mutations will differ, they are also all involved in a common signaling pathway. *CYSLTR2* codes for a seven transmembrane receptor (also termed G protein-coupled receptor), which demonstrates increased activation by the L129Q mutation.[18] The

CYSLTR2 receptor signals through the highly homologous heterotrimeric G proteins Gαq and Gα11, products of the *GNAQ* and *GNA11* genes, respectively. Affecting different downstream signaling pathways, Gαq and Gα11 also activate the product of the *PLCB4* gene, phospholipase-C β4 which hydrolyses PIP2 (phosphatidylinositol 4,5-bisphosphate) releasing DAG (diacylglyerol) and IP3 (inositol-1,4,5-trisphosphate) with subsequent Ca+ release.[19,20] The functional similarity is highlighted by the fact that mutations in these genes were almost always found to be mutually exclusive.[18] As mutations in one of the four genes (*GNA11, GNAQ, PLCB4*, and *CYSLTR2*) were identified in 96% of tumor samples (131 of 136)[18] it appears likely the majority of relevant activating mutations has been identified.

The goal of our study was to investigate the frequency of activating *CYSLTR2* or *PLCB4* mutations in blue nevi. To do this efficiently, a custom-targeted next-generation sequencing approach was applied, assessing all potentially mutated genes in parallel with high sensitivity.

Materials and methods

Sample Selection

Blue nevus samples were obtained by searching the databases of the Department of Dermatology University Hospital Essen (n = 46), Dermatopathology Friedrichshafen (n = 27), and Dermatopathologie bei Mainz (n = 30), Germany. All cases were screened by at least two board-certified pathologists or dermatopathologists. The study was done in accordance with the guidelines set forth by the ethics committee of the University of Duisburg-Essen (IRB-number 16–6951-BO).

DNA Isolation

All DNA was isolated from formalin-fixed paraffin-embedded tissue. Ten micrometer-thick sections were cut from formalin-fixed, paraffin-embedded tumor tissues. The sections were deparaffinized and manually macrodissected according to standard procedures. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Targeted Sequencing

A custom amplicon-based sequencing panel covering 10 genes (Supplementary Table 1) was designed and prepared applying the GeneRead Library Prep Kit from QIAGEN according to the manufacturer's instructions. Adapter ligation and barcoding of individual samples were done applying the NEBNext Ultra DNA Library Prep Mastermix Set and NEBNext Multiplex Oligos for Illumina from New England Biolabs. Up to 60 samples were sequenced in parallel on an Illumina MiSeq next-generation sequencer.

Sequencing analysis was performed applying the CLC Cancer Research Workbench from QIAGEN. In brief, the following steps were applied. The workflow in CLC included adapter trimming and read pair merging before mapping to the human reference genome (hg19). Insertions and deletions as well as single nucleotide variant detection, local realignment, and primer trimming followed. Additional information was then obtained regarding potential

mutation type, known single nucleotide polymorphisms, and conservation scores by cross-referencing varying databases (COSMIC, ClinVar, dbSNP, 1000 Genomes Project, HAPMAP, and PhastCons-Conservation_scores_hg19). The resulting csv files were further analyzed manually. Mutations affecting the protein coding portion of the gene were considered if predicted to result in non-synonymous amino acid changes. Mutations were reported if the overall coverage of the mutation site was ≥ 30 reads, ≥ 5 reads reported the mutated variant and the frequency of mutated reads was ≥ considered 3%.

Associations of Oncogene Mutation Status with Clinical and Pathologic Parameters

We investigated associations of mutation status with available clinical and pathological parameters using χ^2-squared tests and Fisher exact tests as appropriate. All statistical analyses were performed using IBM SPSS Statistics software (version 20.0; International Business Machines, Armonk NY, USA). A *P*-value of $P \leq 0.05$ was considered statistically significant.

Results

Sample Cohort

The study cohort consisted of 103 blue nevus samples from 103 patients (60 females and 43 males) with an average age of 55 years (range 7–91). The tumors included 68 (66%) common, 17 (17%) cellular, 5 (5%) deep seated cellular, 10 (10%) sclerotic, and 3 (3%) amelanotic blue nevi. All samples were primary tumors. Available clinical data are listed in Table 1.

Mutation Analysis for Activating Oncogene Driver Mutations

Targeted amplicon sequencing of all genes as described in the material and methods section was performed for the entire tumor cohort. In line with previous results, the most frequent activating mutations identified were in *GNAQ*, 59% (54c.626A > T Q209L, 6c.626A > C Q209P, and 1c.548G > A R183Q) and *GNA11*, 16% (17c.626A > T Q209L) (Figures 1 and 2). One (1%) *BRAF* (c.1799A > T, V600E) and 3 (3%) *NRAS* mutations (1c.182A > G Q61R, 1c.181C > A Q61K, and 1c.34G > C G12R) were detected. No *PLCB4* mutations were identified. Three tumors (3%) harbored activating *CYSLTR2* c.386T > A L129Q mutations (Figure 3, Supplementary Figures 1 and 2). All mutations were found to be mutually exclusive (Figure 1).

Associations of Clinical and Pathological Parameters with Oncogene Mutation Status

An analysis with available clinicopathological data was performed. The only statistically significant association found was that of oncogene mutation status with cell type ($P = 0.02$). Complete details are presented in Table 1.

Discussion

We analyzed a cohort of blue nevi and found that in addition to previously recognized recurrent activating mutations in *GNAQ* and *GNA11*, mutually exclusive activating hotspot mutations in the *CYSLTR2* gene occur. The mutation type and frequency are essentially

identical to the initial report of *CYSLTR2* mutations in uveal melanoma, once again underlying the genetic similarities between these two melanocytic tumor entities, affecting different organ systems.

Similar to previous reports, we found *GNAQ* mutations to be by far the most common (59%). Frequent Q209L or Q209P mutations with only a rare occurrence of one R183Q mutation is in line with previous data.[10,11] The frequency of *GNA11* mutations with 16% is somewhat higher than the 7.5% frequency (exon 4 and 5 mutations) initially reported in blue nevi.[11] Almost all mutations in both studies were Q209L. The ratio of *GNAQ* to *GNA11* mutations in our cohort is 3.7, which is lower than the initial report of 8.4; however, the clear predominance of *GNAQ* mutations remains well documented.

Our study identified one *BRAF* (V600E) and three *NRAS* (one Q61R, one Q61K, and one G12V) mutations. Neither mutation is frequently found in blue nevi. *BRAF* mutations are frequent in acquired nevi,[9] and *NRAS* mutations in congenital nevi.[21] We excluded cases in our study which were clearly combined nevi, harboring a common nevus cell clone, or cases showing a clear epidermal melanocytic component. The *BRAF*- and *NRAS*-mutant cases in our study were all diagnosed as blue nevi initially and on histological re-review. They showed no apparent epidermal or acquired nevus component. However, the possibility that such regions were present but not represented in the assessed histological slides cannot be entirely excluded. Potentially, future studies will be able to further clarify if presence of *BRAF* or *NRAS* mutations is clearly associated with an epidermal-derived (acquired or combined type) nevus component, leaving *GNAQ, GNA11*, and *CYSLTR2* mutations as genetic markers of truly dermal-derived blue nevi.

The frequency of *CYSLTR2* mutations (3%) in our study is identical to that reported in uveal melanoma.[18] Additionally, all mutations identified were c.386T > A alterations resulting in a L129Q amino acid change, which has been shown to lead to increased receptor activation and signaling output.[18] In conjunction with the genetic evidence of *CYSLTR2* mutations occurring in a mutually exclusive fashion to other known activating mutations (*GNAQ* and *GNA11*) activating the same pathway, this makes a strong case for *CYSLTR2* L129Q mutations being bona-fide driver mutations in the pathogenesis of blue nevi as well as uveal melanoma.

We identified one *PLCB4* D630 mutation using our assay in a cutaneous metastasis of a uveal melanoma (data not shown), but failed to find a *PLCB4* D630 mutation in our cohort of blue nevi. Considering in uveal melanoma, *PLCB4* mutations are rare with a frequency of ~ 4%,[17,18] selection bias may be a factor. Additional studies analyzing considerably larger numbers of tumors will be required before a potential role of activating *PLCB4* D630 mutations in blue nevi can be excluded.

We found no activating oncogene mutation in 17% of blue nevi. The frequency of wild-type tumors (harboring neither a mutation in *GNAQ, GNA11, PLCB4*, or *CYSLTR2*) in uveal melanoma is ~ 5%.[18] There are different potential explanations for the still relatively high number of wild-type tumors in our study. In contrast to uveal melanomas, in which there is generally a considerable amount of available tumor material, blue nevi can be smaller, less

cellular proliferations, frequently interspersed with surrounding connective tissue. This means that the percentage of tumor DNA in the isolated DNA can be low. We applied a next-generation sequencing approach with low frequency mutation cutoffs (3%) to make certain that we detected even low mutation frequencies, in an attempt to compensate for potentially low tumor purity; nevertheless, it is possible that in some of the tumors analyzed and reported as wild-type, the mutation present was not detected due to little or no tumor material obtained in the DNA isolated, or to inadequate sensitivity of our sequencing assay. Another possibility is the presence of additional activating mutations in genes not represented in our assay.

The three *CYSLTR2* mutations we identified in our study occurred in morphologically benign common blue nevi (Figure 3, Supplementary Figures 1 and 2). In such unequivocally benign cases, genetically analyzing tumors to determine oncogene mutation status is not clinically relevant. However, although rare, there are atypical and malignant blue nevi (also termed ‘blue nevus-like melanoma’) that have the potential to metastasize and result in patient death.[5–7] Although the cases are not presented here (manuscript in preparation), we were able to identify and analyze eight tumors of the rare entity malignant blue nevus. No *CYSLTR2* mutations were identified. Considering the low mutation frequency determined in blue nevi and uveal melanoma, higher numbers of these tumors will need to be analyzed to definitively ascertain the presence of *CYLSTR2* mutations in these tumors. In the meantime, it would appear prudent to evaluate *CYLSTR2* mutation status in these tumors, in particular if they have been found to lack mutations in *GNAQ* and *GNA11*. Should *CYSLTR2* mutations prove to be of therapeutic value, identifying such a mutation could potentially be of immediate benefit to affected patients.

Our analysis of oncogene status with clinical and pathological parameters showed a few trends with respect to cell type, age, sex, anatomic site, and pigmentation, although the majority of these associations did not reach statistical significance. Considering five different oncogene mutations were identified, some of them being rare, a considerably larger tumor cohort will be necessary to allow meaningful statistical analyses.

In summary, our findings identify blue nevi as a second tumor type harboring activating mutations in the newly identified *CYLSTR2* oncogene. The mutation frequency of 3% is identical to that in uveal melanoma. *CYSLTR2* mutations being mutually exclusive of oncogenic driver *GNAQ* and *GNA11* mutations in blue nevi is additional supportive evidence of this mutation being a bona-fide driver mutation with clear oncogenic potential. The genetic evidence suggests that similar to activating *GNAQ* and *GNA11* mutations, *CYSLTR2* L129Q mutations may be the only driver mutation present in these tumors and fully sufficient to induce a blue nevus.

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References

1. Zembowicz A, Phadke PA. Blue nevi and variants: an update. Arch Pathol Lab Med. 2011; 135:327–336. [PubMed: 21366456]
2. Zembowicz A, Mihm MC. Dermal dendritic melanocytic proliferations: an update. Histopathology. 2004; 45:433–451. [PubMed: 15500647]
3. Tièche M. Über benigne Melanome (‘Chromatophorome’) der Haut—‘blaue Neavi’. Virch Arch für path Anat. 1906; 1186:212–229.
4. Barnhill RL, Cerroni L, Cook M, et al. State of the art, nomenclature, and points of consensus and controversy concerning benign melanocytic lesions: outcome of an international workshop. Adv Anat Pathol. 2010; 17:73–90. [PubMed: 20179431]
5. Costa S, Byrne M, Pissaloux D, et al. Melanomas associated with blue nevi or mimicking cellular blue nevi: clinical, pathologic, and molecular study of 11 cases displaying a high frequency of gna11 mutations, bap1 expression loss, and a predilection for the scalp. Am J Surg Pathol. 2016; 40:368–377. [PubMed: 26645730]
6. Loghavi S, Curry JL, Torres-Cabala CA, et al. Melanoma arising in association with blue nevus: a clinical and pathologic study of 24 cases and comprehensive review of the literature. Mod Pathol. 2014; 27:1468–1478. [PubMed: 24743221]
7. Martin RC, Murali R, Scolyer RA, et al. So-called ‘malignant blue nevus’: a clinicopathologic study of 23 patients. Cancer. 2009; 115:2949–2955. [PubMed: 19472395]
8. Murali R, McCarthy SW, Scolyer RA. Blue nevi and related lesions: a review highlighting atypical and newly described variants, distinguishing features and diagnostic pitfalls. Adv Anat Pathol. 2009; 16:365–382. [PubMed: 19851128]
9. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. Nat Genet. 2003; 33:19–20. [PubMed: 12447372]
10. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009; 457:599–602. [PubMed: 19078957]
11. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med. 2010; 363:2191–2199. [PubMed: 21083380]
12. Dai J, Tetzlaff MT, Schuchter LM, et al. Histopathologic and mutational analysis of a case of blue nevus-like melanoma. J Cutan Pathol. 2016; 43:776–780. [PubMed: 27152652]
13. Perez-Alea M, Vivancos A, Caratu G, et al. Genetic profile of GNAQ-mutated blue melanocytic neoplasms reveals mutations in genes linked to genomic instability and the PI3K pathway. Oncotarget. 2016; 7:28086–28095. [PubMed: 27057633]
14. Daniels AB, Lee JE, MacConaill LE, et al. High throughput mass spectrometry-based mutation profiling of primary uveal melanoma. Invest Ophthalmol Vis Sci. 2012; 53:6991–6996. [PubMed: 22977135]
15. Martin M, Masshofer L, Temming P, et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. Nat Genet. 2013; 45:933–936. [PubMed: 23793026]
16. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science. 2010; 330:1410–1413. [PubMed: 21051595]
17. Johansson P, Aoude LG, Wadt K, et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. Oncotarget. 2016; 7:4624–4631. [PubMed: 26683228]
18. Moore AR, Ceraudo E, Sher JJ, et al. Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. Nat Genet. 2016; 48:675–680. [PubMed: 27089179]
19. Suh PG, Park JI, Manzoli L, et al. Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Rep. 2008; 41:415–434. [PubMed: 18593525]

20. Waldo GL, Ricks TK, Hicks SN, et al. Kinetic scaffolding mediated by a phospholipase C-beta and Gq signaling complex. Science. 2010; 330:974–980. [PubMed: 20966218]

21. Bauer J, Curtin JA, Pinkel D, et al. Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. J Invest Dermatol. 2007; 127:179–182. [PubMed: 16888631]

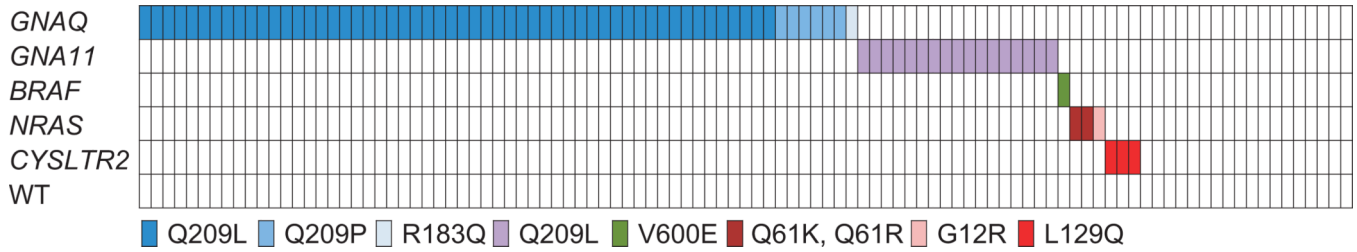

Figure 1.

Distribution of activating mutations identified in blue nevi. Distribution of activating mutations identified in different oncogenes in the blue nevus cohort. The resulting amino acid changes are color-coded according to the scheme underneath the illustration.

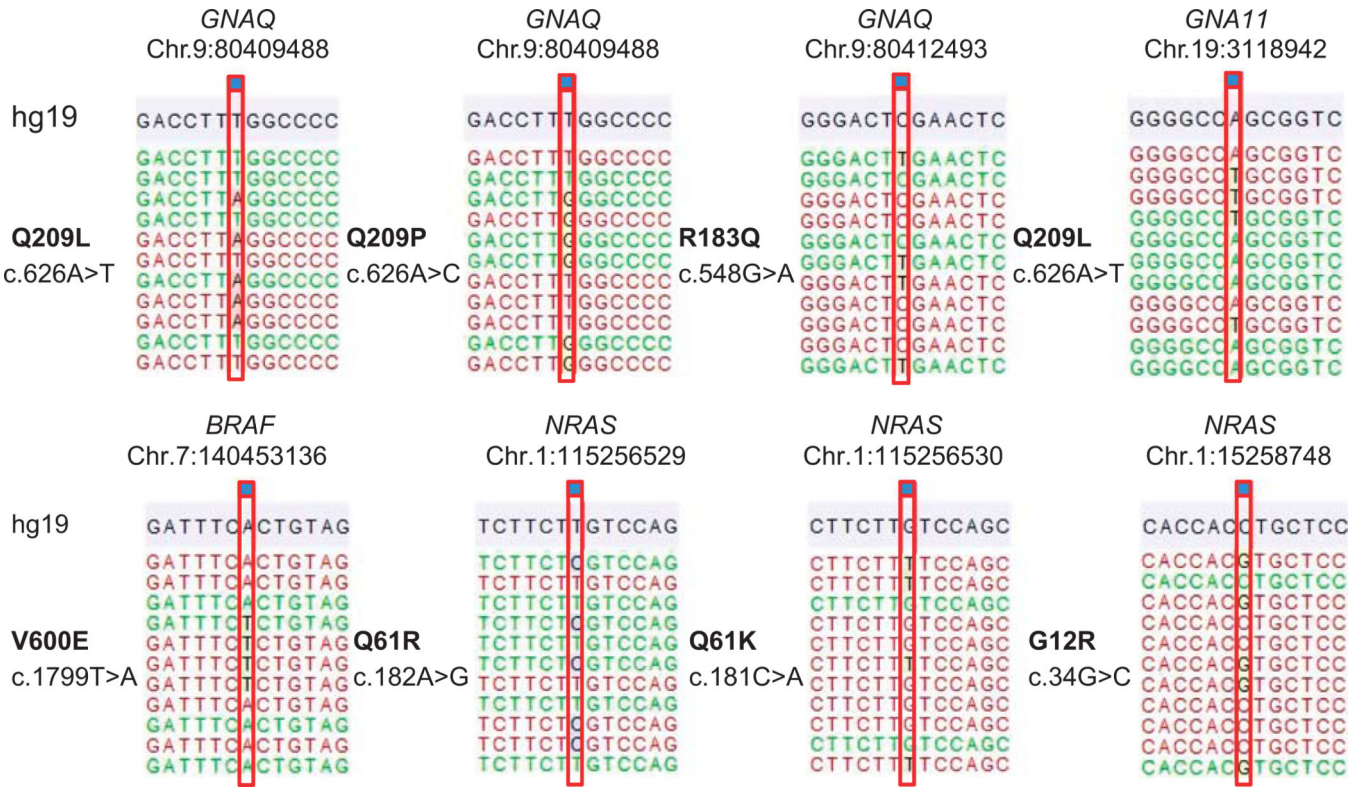

Figure 2.

Examples of activating mutations identified in the tumor cohort. Demonstrated are representative examples of the various mutations in *GNAQ, GNA11, BRAF*, and *NRAS* identified in the analyzed cohort of blue nevi. The notation is according to human genome assembly 19 (hg19). (The depicted *NRAS* mutations show the complimentary strand.)

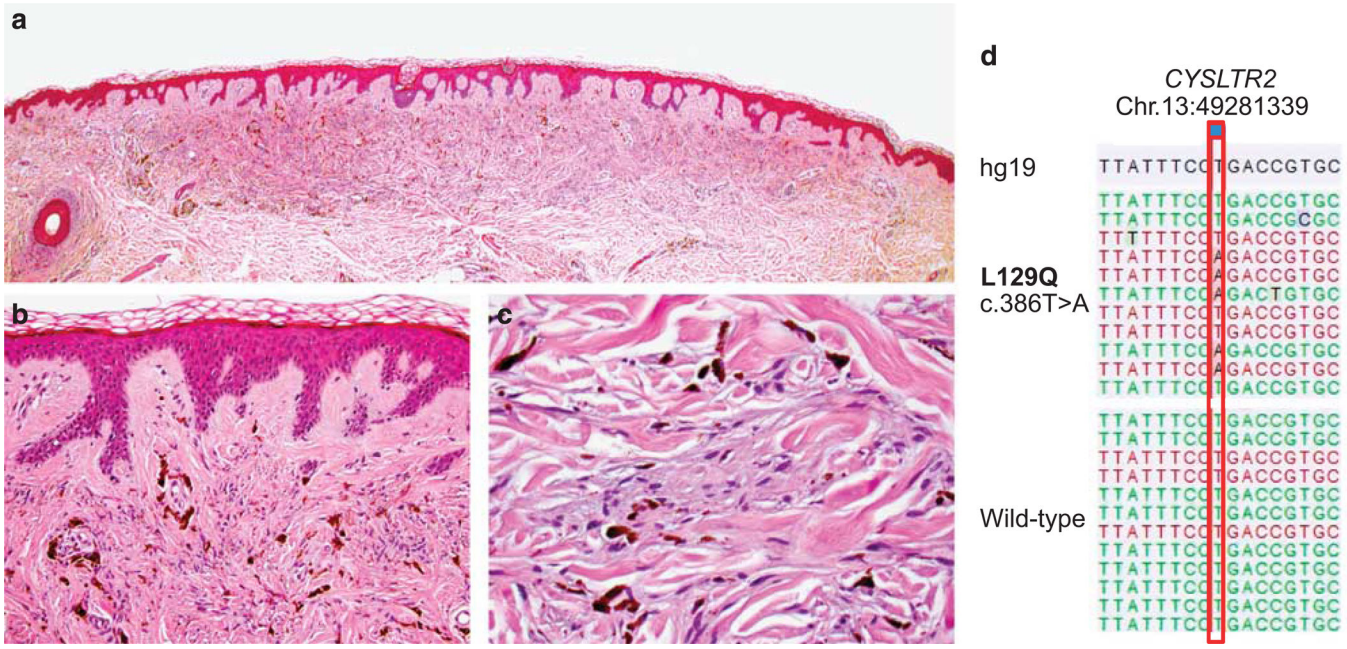

Figure 3.

Activating *CYSLTR2* mutation in a superficial common blue nevus. (**a**–**c**) Histological pictures of a common blue nevus harboring an activating *CYSLTR2* mutation, showing the characteristic proliferation of bland pigmented spindle cells in the dermis with no epidermal involvement ((**a**) ×20, (**b**) ×200, and (**c**) ×400 magnification). The tumor was removed from the upper right arm of a 78-yea-rold male. (**d**) The *CYSLTR2* c.386T > A mutation identified in the tumor with a wild-type sequence for comparison shown underneath. Annotation according to human genome assembly 19 (hg19).

Table 1

Associations of activating gene mutation status with clinicopathologic features

Factor	*Total* N	*WT*	*GNAQ*	*GNA11*	*NRAS*	*CYSLTR2*	*BRAF*	P-*value*
		Median (range)	*Median (range)*	*Median (range)*	*Median (range)*	*Median (range)*	*Median (range)*	
Age	**103**	**41 (17–76)**	**53 (7–91)**	**67 (22–85)**	**70 (31–83)**	**78 (73–81)**	***a***	**0.054**
		N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
Sex								
Female	60	14 (23)	33 (55)	9 (15)	3 (5)	0	1 (2)	0.06
Male	43	4 (9)	28 (65)	8 (19)	0	3 (7)	0	
Anatomic site								
Head/neck	26	6 (23)	13 (50)	5 (19)	0	2 (8)	0	0.08
Upper limbs	27	3 (11)	19 (70)	3 (11)	0	1 (4)	1 (4)	
Trunk	28	7 (25)	19 (68)	1 (4)	1 (4)	0	0	
Lower limbs	17	1 (6)	8 (47)	7 (41)	1 (6)	0	0	
Missing data	5	1	2	1	0	1	0	
Subtype								
Common BN	81	13 (16)	48 (59)	15 (19)	2 (3)	3 (4)	0	0.29
Cellular BN[b]	22	5 (23)	13 (59)	2(9)	1 (5)	0	1 (5)	
Depth								
Upper dermis	11	3 (27)	4 (36)	3 (27)	0	1 (9)	0	0.28
Lower dermis	64	15 (23)	37 (58)	8 (13)	2 (3)	1 (2)	1 (2)	
Subcutis	20	0	16 (80)	3 (15)	0	1 (5)	0	
Missing data	8	0	5	3	0	0	0	
Cell type								
Spindle	77	13 (17)	48 (62)	12 (16)	1 (1)	3 (4)	0	0.02
Epithelioid[c]	7	1 (14)	5 (71)	0	0	0	1 (14)	
Mixed	10	4 (40)	3 (30)	2 (20)	1 (10)	0	0	
Missing data	9	0	5	3	1	0	0	
Pigmentation								
Absent	9	2 (22)	6 (67)	0	0	1 (11)	0	0.06

Factor	*Total* N	*WT*	*GNAQ*	*GNA11*	*NRAS*	*CYSLTR2*	*BRAF*	P-*value*
		Median (range)	*Median (range)*	*Median (range)*	*Median (range)*	*Median (range)*	*Median (range)*	
Age	**103**	**41 (17–76)**	**53 (7–91)**	**67 (22–85)**	**70 (31–83)**	**78 (73–81)**	***a***	**0.054**
		N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
Mild-moderate	70	15 (21)	42 (60)	10 (14)	0	2 (3)	1 (1)	
Marked	15	1 (7)	8 (53)	4 (27)	2 (13)	0	0	
Missing data	9	0	5	3	1	0	0	

[a] 1 patient, age 46 years.

[b] All tumors demonstrating a cellular component

[c] Epithelioid signifies cell morphology with oval to rounded (rather than elongated/spindle) nuclei. Mixed refers to nevi having both a spindle cell and epithelioid component.

WT, wild-type for all tested genes; epith., epithelioid.