The importance of histology

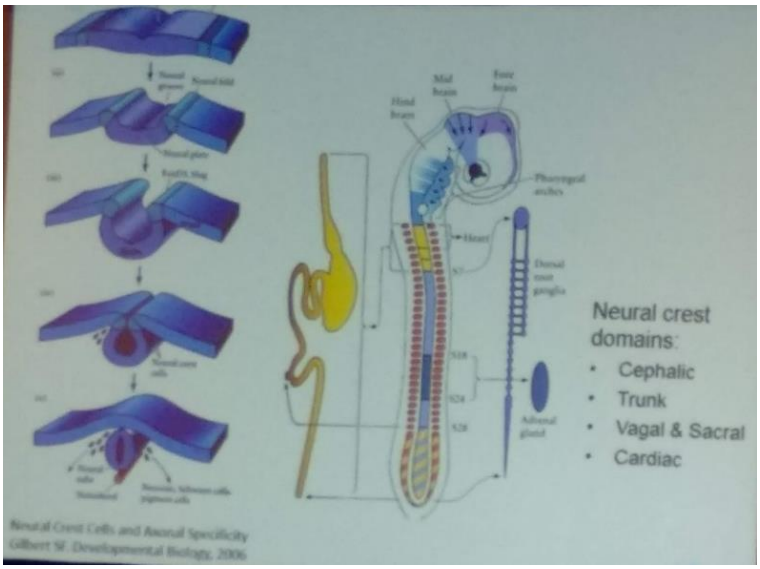
Presentation Miquel Reyes

Nevus Outreach Conference July 2018

Report written by Marjolein van Kessel

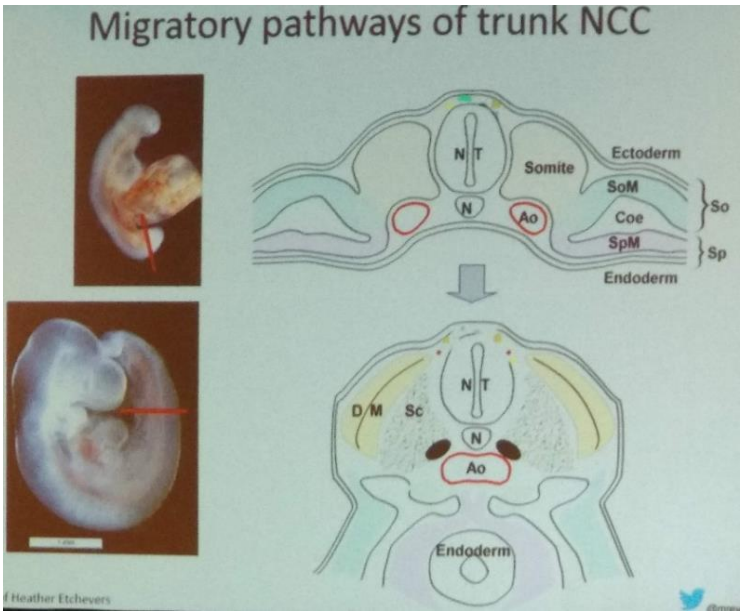
Notification: this report is based on my personal notes and pictures made from the presentation. Though I believe it's quite an accurate report of the presentation, I'm not responsible for misinterpretations or translation errors.

NCM is a neurocristopathy (a diverse class of pathologies that may arise from defects in the development of tissues containing cells commonly derived from the embryonic neural crest cell lineage). It can explain why constipation and NCM can be related. A pathologist will look from inside to the outside (doctors from the outside to the inside). Melanocytes can create frustrated neurons. A mutated cell in the cartilage produces fat or (?). The only interesting thing about vertebrates is the neural crest. He described neurocristopathy.



Epidemiology of melanocytic nevi

In a study of more than 500,000 neonates:
Approximately 1 % of newborns
have a melanocytic nevus.
CMN >10 cm PAS 1:20,000
CMN>40 cm PAS 1:500,000 newborns



Gavin Bailey Tissue Repository

What is the purpose of this Tissue Bank?

Aim:

- To develop a Biological Sample Repository, that will procure, characterize, preserve and selectively distribute biospecimens from patients with L / GCMN NCM.
- The banked biospecimens will be used by our group and collaborating researchers to investigate the molecular mechanisms responsible for the prevention and prevention of these diseases, and will generate findings that allow the development of strategies to treat and/or prevent them.

He talked about ethical issues (samples are de-identified), and consent (permission) has to be given by patients.

Methodology

- The Department of Pathology receives the sample fresh (not in formalin). The pathologist will select the part of the tissue that will be used for diagnosis (not for research).
- Excess tissue from the melanocytic lesion and normal skin tissue (if available) will be sent to the GB bank in:
    - RPMI: a cell culture medium that contains nutrients to keep cells alive.
    - RNA later, a special protectant to preserve RNA

By July 2018- they have collected samples from 188 patients

- Paraffin blocks and HE-stained slides;
- frozen blood (cells and plasma) and fresh tissue
- DNA
- Nevus cells from patients with L / GCMN and NCM

What are the main obstacles?

- Patient/parents wanting to contribute (donate tissue) but finding obstacles from the institution where the surgery is being performed.
- Samples are precious and the institutions want to keep the tissue.
- To obtain more samples from tumors and CNS lesions
- Who "owns" the samples? - The patient !!!!

Conclusions

- BRAF V600E somatic mosaicism, is also associated with L/GCMN. Therefore, NRAS mutations should not be considered exclusive of L/GCMN or NCM (see publication below)
- Nevi with V600E BRAF mutation feature increased extensive dermal/subcutaneous nodules and less hypertichosis (excessive hair growth) than those with Q61 NRAS mutation.
- We confirm the lack of V600E BRAF mutation in Asians, which underscores the significant role played by the racial background in the segregating mutational groups.

Associated problems with Giant Pigmented Nevi

- Risk or malignant transformation (<5%, Genetics?)
- Neurocutaneous melanocytosis (Genetics Pathology?)
- Targeted therapy
- Uncontrollable pruritus (itch) and abnormal scarring (Why what cells / molecules play a role?) (see publication below)
- Psychological problems secondary to cosmetic issues
- Diminished tissue (muscle, vessels, others) growth due to the hamartomatous (cyst?, growth?) nature of the lesions

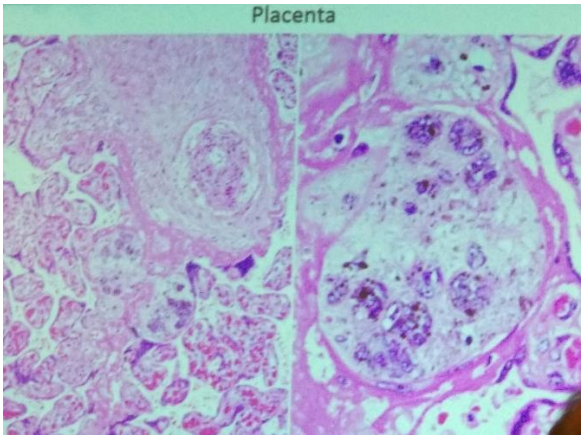
NCM Neurocutaneous Melanocytosis

- is characterized by proliferation or nevus cells in the leptomeninges and brain parenchym.
- it affects 7% of patients with L/GCMN
- NCM may have a benign behavior or remain asymptomatic, but the onset of symptoms generally indicates progression and/or brain, and may develop metastatic dissemination heralding poor prognosis. (see publication below)

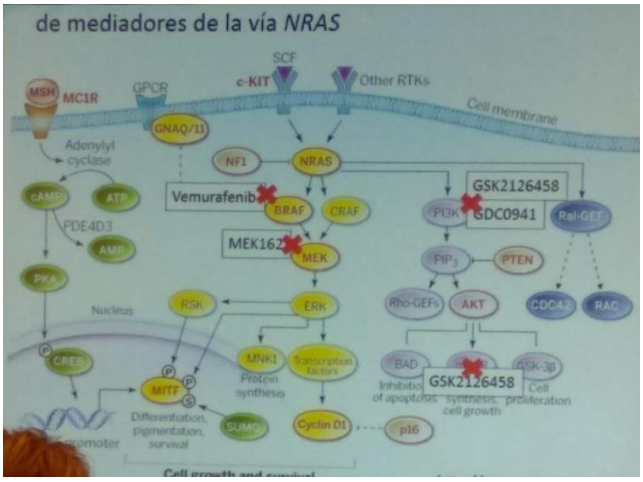
Perineal nevocytoma & NCM

- Patient with Dandy-walker malformation
- Metastatic dissemination of nevus cells through ventriculoperitoneal shunting (see publication below)

Nevus cells have been found in the placenta.



Talking about pathways.
Research to new treatment drugs
in tissue from Gavin Bailey tissue bank
(see publication below)



Publications

BRAF Mutations Are Also Associated with Neurocutaneous Melanocytosis and Large / Giant Congenital Melanocytic Nevi

(http://journals.sagepub.com/doi/abs/10.2350/14-10-1566-OA.1)

Abstract

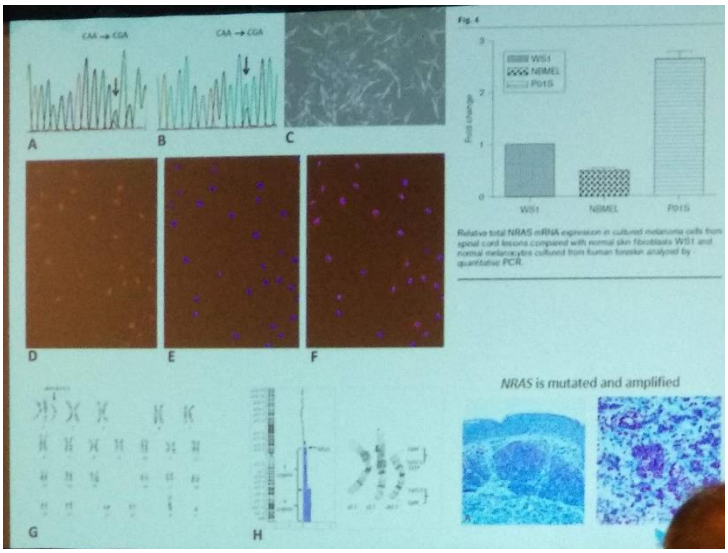
NRAS and BRAF mutations occur in congenital melanocytic nevi (CMN), but results are contradictory. Sixty-six prospectively collected CMN patients were analyzed for NRAS Q61 mutations using Sanger sequencing. Negative cases were evaluated for BRAF V600E mutation. NRAS Q61 mutations affected 51 patients (77.3%), and BRAF V600E was found in 5 (7.6%). NRAS Q61 mutation affected 29 (80.6%) of 36 giant, 16 (80.0%) of 20 large, and 5 (62.5%) of 8 medium-size CMN; BRAF mutation affected 1 (5%) of 20 large and 4 (11.4%) of 36 giant CMN. Compared to NRAS, BRAF-mutated nevi show scattered/extensive dermal and subcutaneous nodules (100% BRAF+ vs 34.8% NRAS+) (P = 0.002). Neurocutaneous melanocytosis (NCM) affected 16 (24.2%) of 66 patients, with NRAS Q61 mutation in 12 (75.0%), and BRAF V600E in 2 (12.5%), P = 0.009. Two patients were negative for both mutations (12.5%). In conclusion, although NRAS Q61 mutations predominate, BRAF V600E mutation also affects patients with large/giant CMN (L/GCMN), and with NCM, a novel finding. BRAF V600E is also associated with increased dermal/subcutaneous nodules. These findings open the possibility of BRAF-targeted therapy in some L/GCMN and NCM cases.

Amplification of mutated NRAS leading to congenital melanoma in neurocutaneous melanocytosis

(https://www.ncbi.nlm.nih.gov/pubmed/26266759)

Abstract

The mechanisms behind malignant progression in patients with giant nevi are largely unknown. Here, we aim to describe novel genetic findings and explain possible mechanisms resulting in the most severe form of neurocutaneous melanocytosis. Detailed histological (biopsy and post-mortem) studies, tissue culture, and high-resolution cytogenetic analysis, including chromosome and array comparative genomic hybridization, Ion AmpliSeq Cancer Panel, and Sanger sequencing, were performed on tissues from a white male who succumbed at 17 months of age to congenital melanoma associated with a bathing-trunk nevus. We also used quantitative PCR to quantitatively assess the expression of NRAS among normal cells, including fibroblast and melanocytes, as well as melanoma cells from our patient. Full autopsy documented tumors in the brain, spinal cord, lung, liver, testis, bone marrow, and, retrospectively, in the placenta. Next-generation sequencing and chromosome microarray in our patient revealed novel findings, including duplication of a mutated NRAS gene, leading to an aggressive clinical course and disseminated disease. Quantitative PCR showed a five-fold increase in NRAS expression in the melanoma cell line when compared with normal melanocytes. Finally, three amino acid-changing germline variants were detected: homozygous TP53 p.P72R, heterozygous KIT p.M541L, and homozygous KDR (VEGFR2) p.Q472H. These genes are involved in malignancy and other potentially relevant pathways, such as mast cell and melanocytic signaling, as well as angiogenesis. These findings provide novel insights into the biology of congenital melanocytic proliferations, showing that amplification of mutated NRAS seems to represent a new genetic mechanism leading to melanoma in the context of neurocutaneous melanocytosis.



Life-threatening blood loss from scratching provoked by pruritus in the bulky perineal nevocytoma variant of giant congenital melanocytic nevus in a child.

(https://www.ncbi.nlm.nih.gov/pubmed/16021164)

Abstract

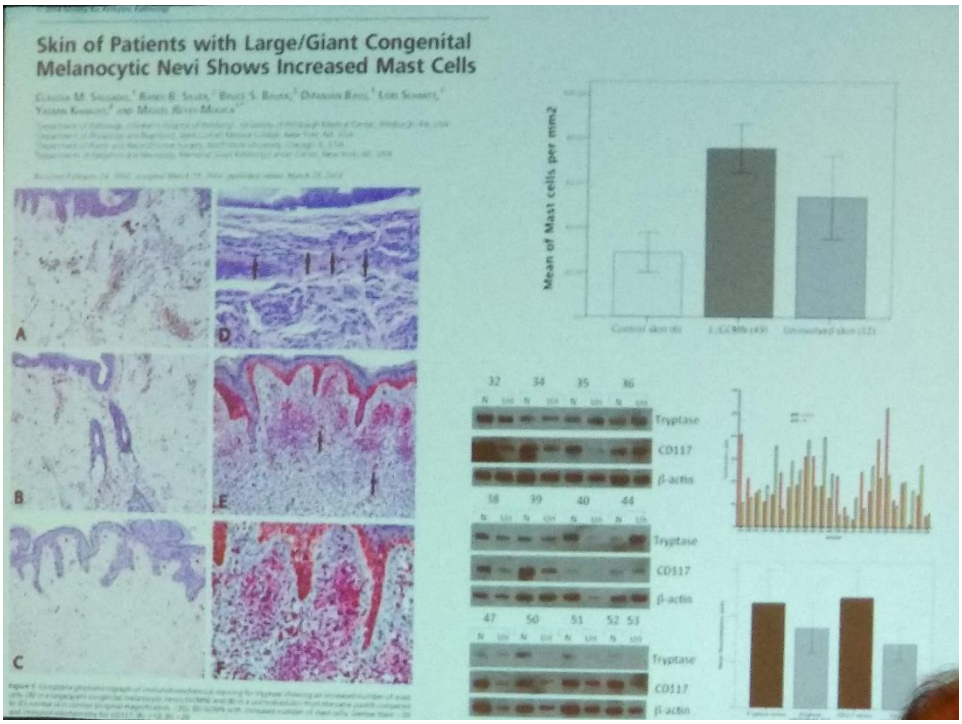
We describe a 3-year-old girl with intractable, debilitating pruritus associated with a giant congenital melanocytic nevus, resulting in life-threatening anemia from extensive bleeding skin excoriations. Multiple conventional oral and topical antipruritic medications failed to provide relief, but the patient was successfully treated with the selective serotonin 5-hydroxytryptamine type 3 inhibitor ondansetron, suggesting a serotonin-related mechanism to her pruritus.

Skin of patients with large/giant congenital melanocytic nevi shows increased mast cells.

(https://www.ncbi.nlm.nih.gov/pubmed/24679055)

Abstract
Nevocytes (NC) and mastocytes (MC) have different progenitors but share stem cell factor as regulator/activator of NC and for differentiation/proliferation of MC. Both cell types express stem cell factor receptor CD117. We hypothesize that large/giant congenital melanocytic nevi (L/GCMN) may associate with MC hyperplasia. Forty-nine L/GCMN were examined, 12 samples from uninvolved skin of L/GCMN patients and 6 control skin samples studied with Giemsa and immunohistochemistry for CD117 and MC-tryptase. Picrosirius red (PR) was used to assess fibrosis. Digital images were used to count MC/mm(2) using ImageJ software. Western blot (WB) for MC-tryptase in 12 GCMN and 12 non-nevus samples was performed. Analysis of variance (Tukey) and Pearson statistical tests were applied. Increased MCs were observed in nevus tissue (75.1 ± 35.3 MCs/mm(2)) and in uninvolved skin (53.74 ± 27.7 MC/ mm(2)). P = 0.109 from patients with L/GCMN, compared with controls from individuals without L/GCMN (28.74 ± 8.4 MC/mm(2)); P = 0.001 supported by results of WB analysis for tryptase. A positive trend toward correlation of MC numbers with fibrosis, assessed by PR staining fell short of statistical significance (r = 0.245; P = 0.086); no difference in fibrosis was found between nevus and non-nevus skin from patients with L/GCMN (P = 0.136). We found a higher density of MC, both in normal-appearing skin and nevus areas of L/GCMN patients, compared with control skin

samples from individuals without nevi. Given the abnormal wound healing and allergic reactions described in L/GCMN patients, these findings suggest a potential role for MC in the biology of L/GCMN, making them a potential target for therapeutic intervention.



Metastatic Peritoneal Neurocutaneous Melanocytosis

https://journals.lww.com/ajsp/Abstract/2008/01000/Metastatic_Peritoneal_Neurocutaneous_Melanocytosis.21.aspx

Abstract
Neurocutaneous melanosis, better referred to as neurocutaneous melanocytosis (NCM), is a rare congenital disorder occurring in childhood characterized by proliferation of melanocytes in the central nervous system (CNS), associated with large congenital melanocytic nevi. The phenotype of the CNS lesions varies, ranging from that of a benign, nevuslike lesion, to one of an aggressive-looking, atypical cell proliferation; however, specific diagnostic criteria allow the distinction from CNS metastasis of a primary skin melanoma. NCM can present with severe neurologic manifestations, and usually has a relentless clinical progression whence neurologic symptoms appear. Dissemination to the peritoneal surface by ventriculo-peritoneal shunting has been exceptionally observed, and we describe 2 cases of such occurrence, one of which was associated with a “bulky perineal nevocytoma” with complex cytogenetic rearrangements. This “metastatic” spreading supports an aggressive phenotype, able to seed and establish new colonies, although only after facilitated translocation of the proliferating cells through the shunt conduit; the aggressiveness of these lesions in our cases is further supported by the histopathologic features and clinical course. The biologic features of NCM cells merit further exploration, as they may shed light on a much more frequent neoplastic neurocristopathy, namely, malignant melanoma.

The Dual PI3K/mToR Inhibitor Omipalisib/GSK2126458 Inhibits Clonogenic Growth in Oncogenically-transformed Cells from Neurocutaneous Melanocytosis.

(https://www.ncbi.nlm.nih.gov/pubmed/29976629)

Abstract
BACKGROUND:
Omipalisib has been found to affect the viability of cancer cells. However, its effect on clonogenicity - a feature of cancer stem cells, is not clear. Cells isolated from neurocutaneous melanocytosis (NCM) patients' lesions grow clonogenically. The aim of this study was to investigate the effect of omipalisib treatment on clonogenic growth of NCM cells in vitro.

MATERIALS AND METHODS:
Clonogenic growth efficiency was evaluated by colony formation assays with or without specific growth factors. Activation of MEK and Akt was determined by immunoblots. Colony formation and cell viability were assessed upon pharmacological inhibition of MEK, Akt and mToR.

RESULTS:
Clonogenicity appeared to depend on bFGF and IGF1signaling through ERK and Akt. Omipalisib treatment prevented colony formation and induced autophagic cell death.

CONCLUSION:
Signaling through Akt is important for survival of clonogenic cells in NCM, and omipalisib treatment as a monotherapy or in combination with MEK162 could be an effective therapeutic strategy to inhibit clonogenic growth.