Genome dynamics and phylogenetic tracking in paired diagnostic and relapsed neuroblastomas

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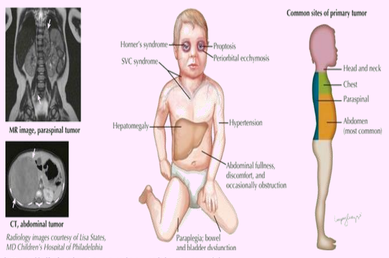
Neuroblastoma



https://clinicalgate.com/neuroblastoma-8/

Neuroblastoma is a cancer of the sympathetic nervous system, and so tumours are found in various regions of the body where certain nervous tissue grows. Tumours are often found in the adrenal glands, neck, chest, abdomen or spine. Symptoms can involve bone pain, abdominal or other site swelling, constipation, weakness due to spinal cord compression, bruising around the eyes, and anaemia.

Key Terms

MYC-N amplification – *Myc-n* is a gene that regulates the expression of many other genes that affect the hallmarks of cancer, and in high risk neuroblastoma this gene is often over-expressed.(1)

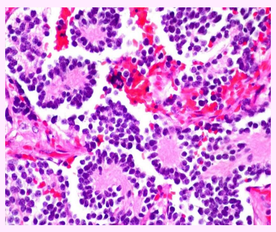
Copy number abnormality (CNA) – a type of structural variation where sections or entire chromosomes exist in multiple copies, and this can affect prognosis of disease.

Copy neutral loss of heterozygosity (CNLOH) –two copies of the same chromosome are inherited from one parent with no copy from the other parent, so total number of chromosomes appears normal.

Chromothripsis – a single event during which thousands of rearrangements at one time in certain regions of a single chromosome, which has been indicted in some cancers.

Variant/ single nucleotide polymorphism (SNP) – a change in a gene that is not prevalent within the majority of the population, and may be associated with disease or predisposition to disease.

Gene cluster – A group of genes that are responsible for producing similar proteins that together complete a generalised function.



Characteristic round blue cells with Homer-Wright rosettes that indicate neuroblastic tumour cells

http://www.pathologyoutlines.com/topic/adrenalneuroblastoma.html

Introduction

- Neuroblastoma (NB) is the 2nd most common solid tumour in children, though in comparison to other childhood cancers, it disproportionately accounts for 15% of paediatric cancer deaths(1).
- Risk stratification informs treatment, and high risk features include: >18 months of age, unfavourable genetics and pathology, metastatic or unresectable disease due to proximity to major structures.
- High risk groups have survival rates as low as 50%, and relapse is frequent, often occurring in 1-2 years after treatment and rarely >5 years later. Relapse is associated with poor prognosis, and survival beyond 1-3 years is rare.
- For this study, particular cases have been selected for genetic analysis due to their atypical relapse time, some of which have occurred up to 18 years later to better understand the changes that may lead to late relapse.

Aims

- To extract, quantify and analyse genetic data from frozen tumours and combine the quantitative data with clinical notes to develop a timeline of genetic events.
- To determine the genetic differences between diagnostic and relapsed tumours to identify potential variants and CNAs that may have lead to atypical/late relapse(s).

Case 1

P29 JW

08/2008

•Suspected typhoid fever at home, later on holiday presented with pallor, abdominal mass, on CT left suprarenal tumour

•Referred to UK hospital with week history of back pain

•Unresectable neuroblastoma, non-MNA with no bony metastasis or bone marrow involvement

•On biopsy poorly differentiated tumour

10/2008

•2 courses of chemotherapy

11/2008

•Surgical resection of primary tumour showed 20% necrosis with large proportion of poorly differentiated

•Relapse 1/post chemo sample obtained

•Chemotherapy and radiotherapy planned

05/2009

•Prior to 3rd cycle - abdominal pain, fever >40 and lethargy

•USS: relapsed neuroblastoma, MIBG positive, unresectable, no tumour available for biopsy

•High dose chemo begun

09/2009

•Extreme blood loss during resection, relapse 2 sample obtained

•Admitted into PICU

•2 weeks post op: back pain - suprarenal mass, MIBG showed active recurrent NB

12/2009

•MIBG scan positive, after high dose chemotherapy

03/2010

•Spiking temperature and abdominal/back pain with distension

•Tumour recurrence, but difference in CNA data to original data on diagnosis.

•Palliative radiotherapy

04/2010

•Died at home

Gene	Time point	Location	Variant
IGFN1	R1	chr1:201178660	Missense
NBPF 1	R1	chr1:16890604	Missense
NBPF 10	R1, R2	chr1:145368499	Missense

•As the table shows, these variants are genes that showed at particular time points that may be targetable in the future.

Methods

Tumours obtained from Children's Cancer & Leukaemia Group (CCLG) Tumour Bank. Aliquots were cut prior to extraction in the lab.

Existing genetic data (whole exome sequencing and SNP arrays) was analysed using specialised software (Nexus and IGV) and results were compared with existing literature

Tumour samples selected due to clinical interest in the atypical relapse(s). Comparative analysis was undertaken to identify novel or confirm known variants/copy number aberrations.

Clinical information where available in detail, was analysed to determine links with the quantitative data and 'case profiles' were made and findings from each summarised.

Case 2

P28

11/1997

•Presented age 4 years with polyuria, back pain, perineal paraesthesia and unsteady gait

•Thoracic tumour found and diagnostic sample obtained

•Induction chemotherapy treatment began

04/1998

•Surgical resection of primary tumour

07/1998

•High dose chemotherapy and post chemotherapy tumour obtained

05/2009

•Neck, lower back and parasternal pain

11/2009

•Liver nodules diagnosed

04/2016

•Presenting with spinal cord compression at site of primary tumour, emergency operation

•Relapse 1 sample obtained, chemotherapy begun

11/2016

•Surgical excision post chemo, 8 weeks in ICU following vena cava thrombosis

•Relapse sample 2 obtained

02/2017

•Back pain, palpable lump, progression of neuroblastoma

•Palliative chemotherapy

Gene	Time point	Location	Variant
NBPF 10	D, PC, R1, R2	Chr1:145297661	Missense
KMT2B	D	chr19:36,208,921-36,229,779	Missense
IGFN1	PC, R1, R2	chr1:201178660	Missense
NBPF 20	R1, R2	Chr1:148344644	Missense
ATP11B	R2	chr3:182554200	Missense
RORA	R2	chr15:60793202	Missense

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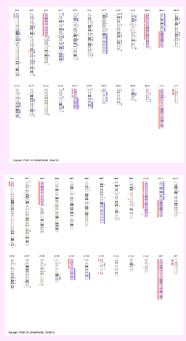
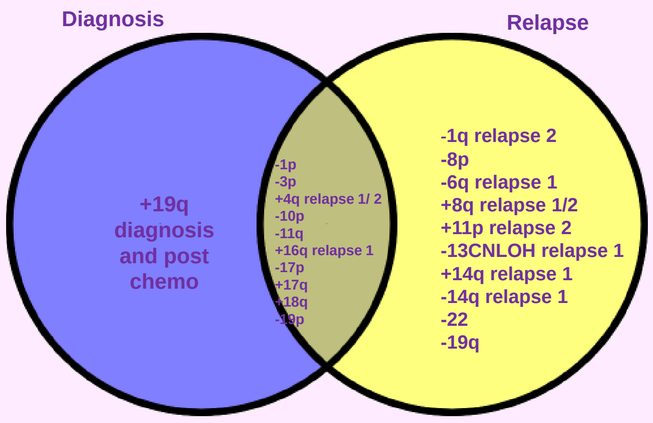


•This image of the Nexus Biodiscovery software shows the patient's copy number aberrations, at diagnosis and relapse 1, which can be compared with the Venn diagram.



Comparison of number of chromosomal abnormalities fro diagnosis to relapse.

Case 3

P32

05/2015

- 19 months old - distended painful abdomen, high heart rate, weight loss and anorexia
- Mass on left side, raised inflammatory markers, and urinary catecholamines – MIBG active unresectable tumour

06-08/2015

- Chemotherapy begun, causing pleural effusion, pain and vomiting
- 3 sites of MIBG positive disease
- High dose chemotherapy and stem cell harvest prior to resection

10/2015

- Resection of adrenal and abdominal neuroblastoma, intraoperative haemorrhage of SMA and coeliac arteries, also pressure ulcer damage to occiput.
- Blood transfusion and admission to PICU

11/2015

- MIBG scan shows faint uptake with no metastatic sites
- High dose chemotherapy started

01/2016

- Radiotherapy begun

02/2016

- Anti-GD2 antibody immunotherapy

08/2016

- Presented with cervical lymphadenopathy, tumour recurrence

06/2017

- Palliative chemotherapy recommended

Gene	Time point	Location	Variant
PCDHB6	R2	chr5:140,529,683-140,532,868	Missense
KMT2C	R2	chr7:151,832,010-152,133,090	Missense

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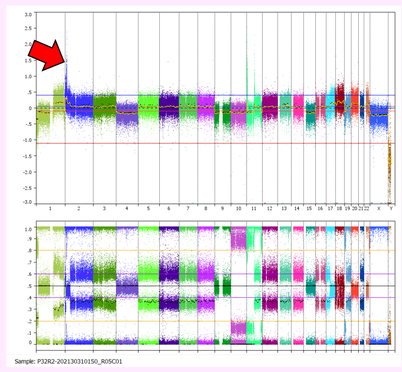
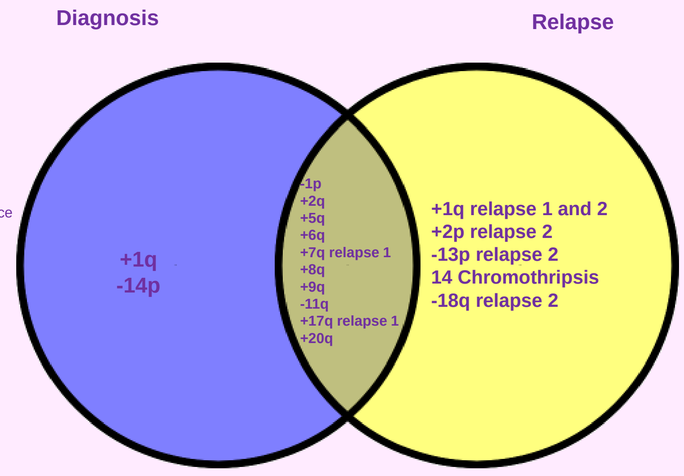


Myc-N amplification can be seen in the first image highlighted by the red arrow on chromosome 2.



Case Findings

- Cases showed many chromosomal abnormalities that were present at diagnosis and at relapse
- Case 1 showed three new genetic variants at relapse that may be of importance; IGFN1, NBPF1 and NBPF10. All three were missense variants on chromosome 1, NBPF genes are in the neuroblastoma break point family of genes, which have been previously indicated in the cancer.
- Case 2 also showed missense mutations in IGFN1 and NBPF10 and NBPF20. NBPF10 was a variant detected throughout every time point, and IGFN1 was detected post chemo and at both relapses. KMT2B, ATP11B and RORA genes were relapse specific.
- Case 3 showed relatively fewer variants of note, including PCDHB6 and KMT2C missense variants, both of which were relapse specific.
- Both cases for which CNA data was available showed substantial chromosomal abnormalities present from diagnosis that then persisted through to relapse. Both cases showed 1p loss, 7q gain, 11q loss and 17q gain from diagnosis to relapse.
- Relapse specific CNAs were also prevalent, both cases showing changes to 1q, 13p and 14q. However, there were no specific gains or losses shared by both.

Conclusions and discussion

- Copy number abnormalities were similar to those documented in literature, though it is interesting that certain abnormalities such as *MYC-N* amplification which is associated with relapse and high risk disease (2), were not seen in high frequencies in the cohort. This could indicate that late or atypical relapse may not have the same pathophysiology as the relapse often associated with *MYC-N* amplification, which could be explored to inform treatment of a potential new sub group.
- 11q deletion which is associated with high risk disease was found in both cases for which CNA data is available.
- *NBPF* gene variants were identified in 2 cases to have persisted from diagnosis to relapse. Variations in this gene family have only been briefly described in literature, so this could be a potential target for new targeted therapies (4)
- Chromothripsis and CNLOH events have been identified from diagnosis to relapse in many cancers (3) and these events were identified within this cohort. This may suggest further exploration into the pathophysiology of these events in neuroblastoma.
- Cases for the study were collected due to the availability of their genetic data and so the patterns and variants identified may not be representative

1) Katherine K. Matthay, J.M.M., Gudrun Schleiermacher, Akira Nakagawara, and Crystal L. Mackall, L.D.a.W.A.W. (2016) 'Neuroblastoma', *Nature Reviews Disease Primers*, 2, 1-21.
2) al, S.e. (2015) 'Mutational dynamics between primary and relapse neuroblastomas', *Nature Genetics*, 47(8), 872-878.
3) Rode A, M.K., Willmund KV, Lichter P, Ernst A (2016) 'Chromothripsis in cancer cells: An update.', *International Journal of Cancer*, 15(138), 2322-2333.
4) Vanessa Andries, K.V., Katrien Staes, Geert Berx, Pieter Bogaert, Gert Van Isterdael, Daisy Ginneberge, Eef Parthoens, Jonathan Vandenbussche, Kris Gevaert and Frans van Roy (2015) 'NBPF1, a tumor suppressor candidate in neuroblastoma, exerts growth inhibitory effects by inducing a G1 cell cycle arrest', *BMC Cancer*, 15(91).