REiNS: Genotype-Phenotype Correlations in Neurofibromatosis and Their Potential Clinical use

Hanemann, Clemens Oliver

http://hdl.handle.net/10026.1/17018

10.1212/WNL.0000000000012436
Neurology
American Academy of Neurology

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.
**REiNS: Genotype-Phenotype correlations in neurofibromatosis and their potential clinical use**

**Author(s):**

Chetan Bettegowda, MD, PhD; Meena Upadhyaya, PhD; D. Gareth Evans, MD; AeRang Kim, MD, PhD; Dimitrios - Mathios, MD; Clemens Oliver Hanemann, MD, PhD on behalf of the REiNS International Collaboration

**Corresponding Author:**

Clemens Oliver Hanemann

oliver.hanemann@plymouth.ac.uk

**Affiliation Information for All Authors:** Chetan Bettegowda, MD, PhD, Johns Hopkins University School of Medicine, Baltimore, USA; Meena Upadhyaya, PhD, Division Cancer and Genetics, Cardiff University, UK; D. Gareth Evans, MD, Genomic Medicine (D.G.E.), University of Manchester, UK; AeRang Kim, MD, PhD, Center for Cancer and Blood Disorders, Children's National Hospital, Washington, DC, USA; C. Oliver Hanemann, MD, PhD, Institute of Translational and Stratified Medicine, Faculty of Health: Medicine, Dentistry and Health Sciences, Plymouth, UK

**Contributions:**

Chetan Bettegowda: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data

Meena Upadhyaya: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data

D. Gareth Evans: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data

AeRang Kim: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data

Dimitrios - Mathios: Drafting/revision of the manuscript for content, including medical writing for content

Clemens Oliver Hanemann: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Number of characters in title: 85
Abstract Word count: 150
Word count of main text: 4215
References: 51
Figures: 0
Tables: 0

**Search Terms:** [ 91 ] All Genetics, [ 92 ] Neurofibromatosis, [ 112 ] Outcome research, [ 214 ] Primary brain tumor, [ 216 ] Nerve tumor
Acknowledgements: The authors acknowledge the support of the Childrens Tumor Foundation for the REiNS International Collaboration

Study Funding: D.G. Evans is supported by the all Manchester NIHR Biomedical Research Centre (IS-BRC-1215-20007). C. Bettegowda is supported by NCI R21 CA208723, DOD W81XWH-16-0078, NCI R37 CA230400, Burroughs Wellcome Career Award for Medical Scientists. C.O. Hanemann is supported by funding from the charity Brain Tumour Research.

Disclosures: C. Bettegowda, MD, PhD is a consultant for Depuy-Synthes; M. Upadhayaya, PhD, reports no disclosures relevant to the manuscript; D.G. Evans, MD, has received travel grants and consultancy fees from Astrazeneca; A. Kim, MD, PhD reports no disclosures relevant to the manuscript; D. Mathios, MD reports no disclosures relevant to the manuscript; C.O. Hanemann, MD, PhD reports no disclosures relevant to the manuscript
Genotype-Phenotype correlations in neurofibromatosis and their potential clinical use

Chetan Bettegowda MD PhD¹, Meena Upadhayaya PhD², D Gareth Evans MD³, AeRang Kim MD PhD⁴, Dimitrios Mathios MD¹ and C Oliver Hanemann MD PhD⁵ for the REiNS International Collaboration

1 Johns Hopkins University School of Medicine, Baltimore, USA
2 Division Cancer and Genetics, Cardiff University, UK
3 Genomic Medicine (D.G.E.), University of Manchester, UK
4 Center for Cancer and Blood Disorders, Children’s National Hospital, Washington, DC, USA
5 Institute of Translational and Stratified Medicine, Faculty of Health: Medicine, Dentistry and Health Sciences, Plymouth, UK

Correspondence: C O Hanemann Peninsula Schools of Medicine and Dentistry, Faculty of Health: Medicine, Dentistry and Human Sciences, John Bull Building, Plymouth Science Park Research Way, Plymouth, PL6 8BU, phone: +441752437418 e-mail: Oliver.Hanemann@plymouth.ac.uk

Word count:
Abstract: 150
Main paper: 4215
References: 51
Tables: 0
Figures: 0

Search terms: Neurofibromatosis, nerve tumour, primary brain tumour, genetics, outcome research

Funding: DGE, is supported by the all Manchester NIHR Biomedical Research Centre (IS-BRC-1215-20007). CB is supported by NCI R21 CA208723, DOD W81XWH-16-0078, NCI R37 CA230400, Burroughs Well come Career Award for Medical Scientists. COH is supported by funding from the charity Brain Tumour Research.
Disclosures: DGE has received travel grants and consultancy fees from Astrazeneca. CB is a consultant for Depuy-Synthes. COH, MU, AR and DM have no disclosures.
Abstract

Objective: As clinically validated biomarkers for neurofibromatosis 1 (NF1) and neurofibromatosis 2 (NF2), have not been identified to date, we wanted to determine whether genotype-phenotype correlations are useful in clinical trials in Neurofibromatosis 1 and 2.

Methods: The biomarker group first performed a systematic literature search and reviewed existing data on genetic biomarkers in NF1 and NF2 and in malignant peripheral nerve sheath tumours (MPNST). The group then met during a series of consensus meetings to develop a joint report.

Results: We found that in NF2 the genetic severity score is clearly of potential clinical use. In NF1, despite over 3000 constitutional variants having been described in the NF1 gene, only four actionable genotype-phenotype correlations currently exist. The diagnosis and treatment decision of these tumours should ideally include histopathology and compilation of some of the genetic markers.

Conclusion: We summarized emerging clinical use of genotype-phenotype correlations in Neurofibromatosis.
The REiNS biomarker remits is to review biomarkers in blood, urine, and tissue for Neurofibromatosis (NF) 1 and 2 and Schwannomatosis. Our previous publication defined biomarker needs in NF1, NF2 and SWN and concentrated on recommendations for protein biomarkers. Here we explore the clinical usefulness of genotype-phenotype relation in Neurofibromatosis 1 and 2. We concentrate on constitutive mutation in NF1 and 2 and in addition discuss somatic mutations in malignant peripheral nerve sheath tumours (MPNST). We explore if mutation analysis could be used to stratify outcomes in clinical trial. In addition, we discuss if some trials should focus selectively on certain genotypes. In the future, mutation analysis may help to select for gene therapy approaches. We also touch on potential MPNST biomarkers resulting from somatic mutations.

**Methods**

The biomarker group first performed a systematic literature search and reviewed existing data on genetic biomarkers in NF1 and NF2. The group then met during a series of consensus meetings to (1) to nominate individual members to summarize the literature in their areas of expertise and assure data comparison between studies and (2) to develop joint report. This report was the circulated to patient representative and REiNS director council for comments.

**Results**

**Neurofibromatosis 2 (NF2)**

The hallmark of NF2 is the development of bilateral, frequently multifocal eighth cranial nerve (vestibular) schwannomas leading to hearing loss and balance disturbance. Schwannomas occur on other cranial, spinal, and peripheral nerve roots and there are also characteristic ‘plaque’ like intracutaneous schwannomas. Meningiomas which are mostly fibroblastic or atypical occur throughout the neuro-axis and are associated with increased mortality. Intraspinal low grade ependymomas also occur and are usually indolent despite their appearances on MRI. In the UK, large population-based estimates of birth incidence for NF2 showed that between 1 in 25-33,000 people would be born with a pathogenic variant in the NF2 gene. Just over 50% of NF2 affected individuals present with no family history, and about a third
to half of these are mosaic for NF2 as the mutation is only present in a subset of cells indicating initial mutation occurred during embryogenesis\(^9\).

NF2 is caused by loss of function mutations in the \(\text{NF2}\) tumor suppressor gene on chromosome 22q\(^4\). Mutations in \(\text{NF2}\) follow the two-hit hypothesis where the first constitutional hit can be different types of mutations from point to large rearrangements, whereas the second hit in the tumour is frequently loss of heterozygosity. Large studies have determined genotype/phenotype correlations with truncating pathogenic variants (nonsense and frameshift) conferring more severe disease courses than missense mutations, splice site mutations or large deletions\(^{10, 11}\). In addition the position of the mutation correlates with mutations in the 3’ end of the gene (exons 14/15) being associated with fewer meningiomas\(^{12}\) and lower mortality. Mosaic affected individuals have a milder form of NF2, consistent with less cells carrying the pathogenic variant\(^9, 13\).

A study in 142 UK patients led to the suggestion of a genetic severity score using the following genotype-phenotype correlations:\(^{13}\)

- **Type 1:** Mild: mosaic for mutations only found in tumour, not blood
- **Type 2a:** Mild: missense variants, exon 1 and 13/14 truncating, Splicing 7-15, mosaic for variants except in 2b in blood
- **Type 2b** Moderately severe: Large deletions, Splicing variants exons 1-6, mosaic for truncating variants (exons 2-13) in blood.
- **Type 3:** Severe: Truncating mutations exons 2-13

Type 3 variants are associated with very early mortality with almost no one living beyond 60 years of age\(^6\). These are associated with frequent childhood onset\(^{14}\) and high frequency of meningiomas. Type 2b are intermediate with significantly better survival than type 3\(^6, 11\), but more severe than type 2a. The classification also accounts for later and milder disease caused by mosaic variants that are not found in blood analysis\(^9, 13\). Accordingly, there is a statistically significant but weak correlation of the genetic severity score with quality of life and number of interventions\(^{13}\).
A follow up study showed that genetic severity is a significant predictor of hearing outcomes including Optimum Discrimination Scores (ODS), hearing classification, and maximum annual pure tone average (PTA) deterioration. Median age of serviceable hearing varied from 32 to 80 years depending on genetic severity\textsuperscript{15}. The authors use a mild genetic severity score in counseling the patient in clinic. Nonetheless, age of NF2 individuals needs to be taken into account especially in those with pre-symptomatic testing.

**Neurofibromatosis 1 (NF1)**

Identification of a specific *NF1* variant cannot generally predict the progression or outcome of the disease in an NF1 patient, even within a family. It is important to note that the development of many NF1 related tumors including that of MPNST, one of the most lethal manifestations of NF1, is a two hit phenomenon. Phenotype is regulated by multiple factors including age dependent manifestations, the timing and number of second hits in specific cells, allelic and non-allelic heterogeneity, cellular heterogeneity, epigenetics, modifying loci, environmental and stochastic factors. It is the interplay of all these factors which determines a specific phenotype. Identification of a genotype-phenotype correlation for a particular constitutional variant or variant type aids in the clinical management and genetic counselling of patients. However, although more than 3197 different constitutional *NF1* pathogenic variants have been identified (HGMD www.hgmd.cf.c.uk/), only four clinically confirmed genotype phenotype correlations have been reported, relevant to 10-15% of the NF1 population.

**Germline genetic modifications contributing to the genotype-phenotype correlation in NF1**

*NF1* p.Met992del

This genotype phenotype correlation was described in 2007 involving the in-frame deletion of codon 992: p.Met992del\textsuperscript{16}. Patients with this variant have a milder phenotype primarily comprising café-au-lait spots and skinfold freckling, and lack cutaneous and visible plexiform neurofibromas, which are the hallmark features of NF1 (see also D Wallis this issue). The study cohort in this international study included 21 patients (14 familial and 7 sporadic) and 26
affected relatives. The other clinical features described in this cohort of p.Met992del patients include learning problems (17%), pectus anomalies (16%), short stature (11%), scoliosis (10%), pulmonary stenosis (9%), macrocephaly (9%), and symptomatic spinal neurofibroma (2%). A subsequent larger study confirmed these findings, and also failed to find external visible plexiform or cutaneous neurofibromas. Unlike the previous study, 4.8% of individuals were found to have non-optic pathway tumours, but they were mostly low-grade and asymptomatic. A higher proportion (38.8%) had cognitive impairment/learning disabilities, compared to the 17% reported. The overall prevalence of lipomas in individuals with p.Met922del in both studies combined was 5.5%. The molecular mechanism associated with this mutation remains unknown. The frequency of the p.Met992del variant in NF1 mutation–positive unrelated individuals in the NF1 Leiden Open Variation Database (LOVD) is 0.78% (27/3442) and in the University of Alabama (UAB) cohort is reported to be 0.88% (74/8400).

This mild phenotypic spectrum overlaps with the clinical features observed in Legius syndrome which is caused by pathogenic variants in SPRED1. However, patients with Legius syndrome are distinct from those with NF1 in not having Lisch nodules.

**NF1 p.Arg1809**

This genotype phenotype correlation was first reported by involving a missense change at codon 1809, an arginine residue which is highly conserved and located in the plekstrin homology (PH) domain of neurofibromin. Six unrelated NF1 patients with p.Arg1809Cys, due to NF1 c.5425C>T exhibited café-au-lait spots and freckling, macrocephaly, thoracic abnormalities, reduced growth, and learning problems. Notably similar to p.Met992del, these patients did not have discrete cutaneous, spinal and plexiform neurofibromas, optic pathway gliomas, other malignancies, or skeletal abnormalities. These findings were confirmed by a multi-centre comprehensive study. In approximately 25% of the individuals Noonan-like features could be found. Pulmonic stenosis and short stature were significantly more prevalent compared with classic cohorts (P < 0.0001). In over 50% of patients developmental delays and/or learning disabilities were reported. Melanocytes cultured from a CAL in a segmental NF1-patient showed two different somatic NF1 mutations, p.Arg1809Cys and a multi-exon deletion, providing genetic
evidence that p.Arg1809Cys is a loss-of-function mutation in the melanocytes and causes a pigmentary phenotype. Constitutional missense variants at p.Arg1809 are reported in ~1.23% of unrelated NF1 probands: 0.87% (30/3442) in the NF1 LOVD and 1.23% in the UAB cohort\textsuperscript{18,21}.

We suggest that patient/families with the above-named mutations are not included in a natural history studies or clinical trials investigating plexiform neurofibromas.

**NF1 microdeletion**

About 4.7 – 11% of NF1 patients have a so-called ‘microdeletion’ of 14 protein coding genes including NF1 and four micro RNA genes. Three different size NF1 microdeletion have been reported\textsuperscript{22}. The commonest type of NF1 microdeletion, accounting for 70-80% of such cases is type 1, which spans 1.4 Mb and is estimated to occur with a frequency of 1 in 60,000\textsuperscript{22}. Most type 1 NF1 microdeletions are caused by inter-chromosomal non-allelic homologous recombination (NAHR) during maternal meiosis. The NAHR is facilitated by the presence of recurrent breakpoints in low-copy repeats-NF1-REP\textsubscript{a} and NF1-REP\textsubscript{c}.

Type 2 NF1 microdeletions encompass 1.2 Mb and are associated with hemizygosity of 13 protein coding genes, including LRRC37\textsubscript{B}. They are caused by mitotic rather than meiotic NAHR and hence are associated with somatic mosaicism and a less severe phenotype\textsuperscript{23}. The breakpoints of type 2 deletions map to SUZ12 and its pseudogene SUZ12\textsubscript{P1}, which flank NF1-REP\textsubscript{c} and NF1-REP\textsubscript{a}. At least 10% of NF1 microdeletions are type 2.

Type 3 NF1 microdeletion encompasses 1.0 Mb and account for 1-4% of all patients with large NF1 deletions. In contrast to type 1 microdeletions, type 3s do not include the five functional genes CRLF3, ATAD5, TEFM, ADAP2 and RNF135. Only 10 NF1 patients with 1.0 MB deletion have so far been reported. Cognitive impairment was observed in only 50% (4/8 patients). Type 3 microdeletions are mediated by NAHR between NF1-REP\textsubscript{b} and NF1-REP\textsubscript{c} leading to hemizygosity of nine protein-coding genes.
Type 4 microdeletions are unusual in that they are not associated with recurrent breakpoints and thus have a variable number of genes in the deleted region. Type 4 microdeletions can be both constitutional and post-zygotic. It is estimated that these constitute 8-10% of all large deletions.

NF1 patients with type 1, 1.4 Mb deletions, exhibit a more severe phenotype. These patients have increased numbers of cutaneous, subcutaneous, plexiform and spinal neurofibromas as compared with general NF1 population. They also have an extremely high burden of internal neurofibromas. They have four-fold increased risk of MPNST. Co-deletion of SUZ12 or EED gene in addition to NF1 further increases MPNST risk and hemizygosity of ATAd5, COPRS, UTP6 and RNF135 also contribute to increased tumour risk. Complete loss of PRC2 (SUZ12, EED) function in plexiform neurofibroma derived from microdeletion patients is important for malignant transformation to MPNSTs. In addition, they have dysmorphic facial features, are tall for their age and exhibit other features of overgrowth, such as large hands and feet, hyper flexibility of joints, skeletal abnormalities and muscular hypotonia. They are associated with impaired cognitive development and increased cardiovascular anomalies as compared with the general NF1 population. Loss of RNF135 in the micro-deleted region is considered to be the cause of the dysmorphic facial features and overgrowth.

Somatic mosaicism for type 1 microdeletions is rare: only three such patients have been reported, two of these patients exhibited general manifestation of NF1 and the third had segmental NF1. All three had a milder phenotype than that seen in typical type 1 microdeletion patients. Overall clinical severity of the microdeletion patients is determined by the size of the deletion and somatic mosaicism.

Missense mutations in NF1 codons 844-848

The fourth genotype phenotype correlation is with missense mutations affecting one of the five codons 844 – 848 in the Cysteine-Serine Rich (CSR) domain, which is associated with a severe phenotype. This study included 129 unrelated probands and 33 affected relatives. These patients have a high prevalence of plexiform and/or spinal neurofibromas, symptomatic and asymptomatic optic pathway gliomas OPGs, malignant neoplasms, and skeletal abnormalities.
This severe phenotype was observed in 75% of adult NF1-affected individuals with these variants in codons 844 – 848, clearly demonstrating that missense mutations outside the GTPase-activating Protein-related domain (GRD) can be associated with a severe clinical presentation. 25% of NF1 subjects with such variants do not have a typical severe phenotype. Missense and single amino acid deletions can be less detrimental as they alter only a discrete region of protein and perhaps impact protein function in a more precise manner.

Focusing on the recurrent and highly conserved missense variants may provide more predictive markers. Four clear genotype phenotype correlations have been identified so far, offering biomarkers for clinical management and genetic counselling. Notably, each of the genotype phenotype correlations only affects a small percentage of NF1 individuals, 5.9% with microdeletions, 0.78% with p.Met992del, ~0.9 - 1.2% with p.Arg1809 missense variants and 1.6% with missense variants at codons 844 – 848. Taken together, therefore, approximately 10% of NF1 patients can be counselled more specifically about the likely progression of certain aspect of their disease. Patients and families with p.Met992del and p.Arg1809 missense variants should likely not be included in natural history or clinical studies investigating plexiform neurofibromas as these manifestation do not occur in this small subset. Although one has to take into account that plexiform neurofibromas are congenital and frequently detected by imaging especially when whole body MRI is done routinely. We are just beginning to unravel relationship between specific variants or types of variants and clinical features for NF1 patients after nearly 30 years of study. Availability of large number of clinically and molecularly well characterised NF1 patients contributed by multiple genetic centres will pave the way for future genotype phenotype correlations.

Other NF1 genotype phenotype correlations which have not been confirmed in larger datasets are described below.

Missense or splice-site NF1 mutations - Familial Spinal Neurofibromatosis (FSNF)
Spinal tumours that develop in classical NF1 patients usually occur in small numbers and only affect one region of the spine, with most symptomatic tumours situated below the cervical level.
The NF1 constitutional variant spectrum associated with such patients is typical of that observed in the general NF1 population. In contrast, patients with familial spinal neurofibromatosis (FSNF) present with multiple bilateral spinal tumours involving large regions of the spine, frequently causing symptoms resulting from cervical spinal cord compression. Despite these symptomatic tumours, these patients exhibit few if any other NF1 clinical features. A number of FSNF families have been reported and their constitutional NF1 variants studied. The risk of having FSNF vs classical NF1 was significantly increased in individuals harbouring missense or splice site variants.

Breast Cancer
In a cohort of 78 NF1 patients with breast cancer, it was highly significant that no cases were observed with either partial or whole gene deletions (p = 0.014), suggesting that microdeletion patients are not at increased risk of breast cancer (HR 0.11). While no overall correlations were observed between other variant types and the risk of breast cancer, 45 (64.3%) of the 70 different variants observed were enriched, i.e. were observed more frequently than expected, with p values between 0.001 – 0.049 and associated hazard ratios of 6.4 – 83. In addition, a higher proportion of nonsense variants were observed in association with breast cancer over the age of 50 years, and 90% (10/11) of those with missense variants and known age of onset of breast cancer occurred under 50 years (p = 0.041). These findings require confirmation in a larger independent cohort and currently will be up to individual clinicians to decide on actionability.

Somatic genetic changes and epigenetic modifications contributing to phenotypic variation in NF1 patients
The progression from a normal Schwann cell to an MPNST is a phenomenon that requires multiple genetic and epigenetic changes to be orchestrated under a supportive microenvironment. In the majority of cases, an NF1 patient will initially develop a plexiform neurofibroma that over time will transform to an MPNST. The second hit in the process of MPNST formation in NF1 patients is somatic mutations acquired at the level of haploinsufficient
(NF1+/-) Schwann cells that lead to additional deletion or activation of key genes important in cancer related pathways. In the majority of the cases, neurofibromas are very distinguishable from MPNST that exhibit increased cellularity, increased mitosis, cytological atypia, and sometimes necrosis. However, there are cases that show mixed features of lower grade and higher grade and they are hard to classify. This group of neurofibromas is collectively called atypical neurofibromas (ANF). Of these some will remain benign over time whereas others will progress to MPNST within a few years from initial diagnosis. A recent classification motif groups the latter under the term “atypical neurofibromatous neoplasms of uncertain biologic potential” (ANNUBP) to indicate the greater risk these ANF have for transformation to MPNST.

Somatic mutations in atypical neurofibromas and MPNST
Somatic mutation burden and genomic instability in ANF is comparatively low, with only NF1, CDKN2A/B and, to a lesser extent, SMARCA2 mutated in the tumors. SUZ12, EED or TP53, which are frequently inactivated in MPNST, are not mutated in ANF. Comparing unmatched neurofibromas versus MPNST from pooled NF1 population demonstrates loss of CDKN2A/B appears to be the main genetic event that in addition to NF1 inactivation leads to premalignancy. The transition to MPNST coincides with a rise in genomic instability, inactivation of PRC2 complex genes such as SUZ12, EED or KDM2B and copy-number gains of cell cycle and pluripotency genes.

A longitudinal analysis of patients with NF1 from diagnosis with a neurofibroma to the transformation to an MPNST has the advantages to analyze the spatial and temporal mutations of neurofibromas in these patients. Hirbe et al. performed whole exome sequencing in an NF1 patient that had progression of a lesion from plexiform neurofibroma to MPNST and metastasis and identified an increasing number of cells with somatic inactivation of NF1 during progression of the disease. Additionally they identified loss of one copy of TP53 in the MPNST and its metastasis but not in the plexiform neurofibroma.

DNA methylation/Histone modifications in the progression from neurofibroma to MPNST
Multiple studies demonstrate that the transformation from plexiform neurofibroma to MPNST is an epigenetic phenomenon. Specifically, loss of SUZ12, EED or KDM2B genes in MPNST inactivates the PRC2 pathway responsible for methylation of the lysine 27 of histone H3 leading to hyperactivation of multiple key cancer related and developmental pathways. Development of MPNST in NF1 patients may be a three hit phenomenon where NF1 is lost with SUZ12 as part of the microdeletion syndromes as a first hit and consequently somatic NF1 loss as a second hit with a final hit being the loss of the remaining final SUZ12 copy leading to complete inactivation of the PRC2 complex. Immunohistochemistry (IHC) of MPNST demonstrate decreased levels of 5mC, 5hmC and H3K27me3 in MPNST compared to plexiform neurofibromas and dermal neurofibromas. Hypermethylation of CDKN2A, WT1 and S100B is frequent in MPNST compared to neurofibromas in human samples. Methylome profiling of Schwann cells, neurofibroma and MPNST from patients with NF1 using Methylated DNA Immunoprecipitation Sequencing (MeDIP-seq) technology showed that there was no significant global hypomethylation in MPNST compared to neurofibromas or Schwann cells in contrast to what has been reported for other tumor types. However, satellite repeats showed a highly significant directional difference in DNA methylation, suggesting these repeats represent the main target for hypomethylation in MPNST. The functional significance of this pattern of hypomethylation in the repeat regions of MPNST genome remains unclear. In addition, a key number of genes in MPNST are identified as being hypermethylated, driving a suppressive effect of RNA expression of these genes. For example the CpG island of the promoter region of SOX10 and CDKN2A were highly hypermethylated in MPNST compared to neurofibromas or Schwann cells leading to decreased gene expression.

Application of genetic data in diagnostics and prognostication of MPNST

Despite many efforts and the significant increase in the amount of genetic information known about MPNST, there is still no blood based or tumor based genetic marker that can distinguish with certainty the transition of a neurofibroma to MPNST. As a result, the diagnosis of MPNST is made based on careful analysis of the whole tissue given for histopathology and compilation of some genetic markers.
One promising marker that can help in the diagnosis of MPNST is the assessment of H3K27me3 by IHC. Loss of H3K27me3 points to the diagnosis of MPNST, however the presence of H3K27me3 does not exclude the diagnosis of MPNST. Schwann cell markers (S100, Sox10) are often lost in MPNST. Loss of CDKN2A as mentioned above differentiates plexiform neurofibromas from ANF and MPNST but may not differentiate the two entities. TP53 intense positivity points to MPNST.

There are very few specific genetic aberrations identified as prognostic markers for survival in MPNST. RASSF1 promoter methylation was associated with decreased survival in patients with NF1 associated MPNST, but this difference in survival was not noted in sporadic MPNST patients. Hypomethylation of the MPNST specimens was associated with increased RNA expression of the RASSF1 gene. RASSF1 gene is important in regulation of microtubule formation and is therefore conceivable that decreased expression of the gene can lead to genomic instability that is associated with higher grade lesions. ATRX protein expression is an NF1 specific prognostic marker of survival in MPNST, but does not appear to be correlated in sporadic cases. ATRX is a gene that regulates telomere lengthening and its loss leads to immortalization of tumor cells. It can additionally affect the PRC2 complex to regulate methylation of histones leading to regulation of key developmental and cancer related pathways. ATRX mutations have a well-established role in gliomagenesis and progression of glial tumors and many other malignancies.

**Discussion**

In summary, genotype phenotype correlations in humans are complex, as phenotype is neither homogeneous nor perceptible. With the advent of NGS, the vast genetic variations reflected in the form of SNP, polymorphism, frameshift insertion and deletions, CNVs and triplet repeats may be good predictors.

In NF2 the genetic severity score is clearly of potential clinical use. Clinical trials will need to adjust for genetic severity. Ideally, any randomisation should stratify by age and genetic severity category. Early phase trials should probably be confined to type 2b and 3.
In NF1 despite over 3000 constitutional variants having been described in the *NF1* gene, only four actionable genotype phenotype correlations currently exist. A diagnosis of NF1 can be confidently made in a majority of patients by using the clinical diagnostic criteria supported by molecular tests. Although information on *NF1* germline mutations can be easily achieved, we still lack sufficient knowledge of the regulatory and unlinked genetic factors. As only very few variants can predict the severity and progression of the disease, many women from NF1 families do not opt for prenatal testing because the severity of disease cannot be accurately predicted on an individual basis. Therefore, additional biomarkers for genotype phenotype relationships are needed. With increasing knowledge of MPNST pathogenesis, the diagnosis and treatment decision of these tumours include histopathology and compilation of some of the genetic markers.

The paucity of well characterised genotype phenotype correlations may be due to the marked genetic heterogeneity seen in NF1 patients, lack of variant clustering and that a majority of constitutional variants are private. Clinical manifestations are often age dependent, therefore, it is imperative children also be included in future studies. Other hampering factors include observed intra- and interfamily clinical variability, mosaicism in the founder member, multiple modifying loci and environmental factors. In addition, without functional analyses, one cannot be absolutely confident about the pathogenicity of a non-recurrent missense variant. Comprehensive clinical details are required for each patient, but in a busy clinic this can be a challenging task for a physician. The Human Phenome project, which requires phenotype data to be recorded in a systematic way, as has been done in Decipher\(^{50}\) and the 100KGP [https://doi.org/10.6084/m9.figshare.4530893.v5 2019], will further aid the analysis of genotype phenotype correlations.

Human Phenotype Ontology\(^{51}\) allows machine searchable description of phenotype. By integrating data on DNA variants into knowledge networks and reasoning them with Artificial Intelligence (AI) could help define deep genotype. AI could also be useful in predicting genotype phenotype correlation by deep phenotype of the clinical information from the electronic health
records (EHR) and integrate that with genomic data. Deep learning methodologies have also been employed to predict sequence specificity of DNA and RNA binding proteins.

Extensive research in the genetic and epigenetic analysis of NF1-related tumors show the significance of DNA methylation and histone modifications, as well as the accumulation of somatic mutations through the progression of benign to malignant tumors, as important factors contributing to the development of phenotypic features that cannot otherwise be explained by germline genetic aberrations. Identification of tumors early in the life of these patients, in particular in those with high risk of developing NF1 associated tumors, is important. For these patients, recommended follow up via imaging modalities such as whole-body MRI and multi-disciplinary NF clinics is required for timely and accurate diagnosis and management. It is hoped that continued understanding in the mechanisms of genetic aberrations in tumor development will lead to preventative and treatment methods for patients with NF1 and its associated tumors.

It is pertinent that all health care workers dealing with NF patients are updated on the established and emerging genotype phenotype correlations. High throughput technology including NGS and WES is revolutionising clinical research, leading to novel drug development and paving the path to precision medicine. We anticipate that improved genotyping and phenotyping methods combined with prudent approaches will aid us to understand the complexity of the gene, the underlying molecular mechanisms and heterogeneous phenotype of NF patients.

Acknowledgements:
The authors acknowledge the support of the children’s tumor Foundation for the REINS International Collaboration
<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chetan Bettegowda</td>
<td>Johns Hopkins University&lt;br&gt;School of Medicine, Baltimore, USA</td>
<td>Major role in the acquisition of data&lt;br&gt;Analysis or interpretation of the data&lt;br&gt;Drafting or revising the manuscript for intellectual content</td>
</tr>
<tr>
<td>Meena Upadhayaya</td>
<td>Division Cancer and Genetics, Cardiff University, UK</td>
<td>Major role in the acquisition of data&lt;br&gt;Analysis or interpretation of the data&lt;br&gt;Drafting or revising the manuscript for intellectual content</td>
</tr>
<tr>
<td>D Gareth Evans</td>
<td>Genomic Medicine, University of Manchester, UK</td>
<td>Major role in the acquisition of data&lt;br&gt;Analysis or interpretation of the data&lt;br&gt;Drafting or revising the manuscript for intellectual content</td>
</tr>
<tr>
<td>AeRang Kim</td>
<td>Center for Cancer and Blood Disorders, Children's National Hospital, Washington, DC, USA</td>
<td>Major role in the acquisition of data&lt;br&gt;Analysis or interpretation of the data&lt;br&gt;Drafting or revising the manuscript for intellectual content</td>
</tr>
<tr>
<td>Dimitrios Mathios</td>
<td>Johns Hopkins University&lt;br&gt;School of Medicine, Baltimore, USA</td>
<td>Drafting or revising the manuscript for intellectual content</td>
</tr>
<tr>
<td>C Oliver Hanemann</td>
<td>Institute of Translational and Stratified Medicine, Faculty of Health: Medicine, Dentistry and Health Sciences, Plymouth, UK</td>
<td>Design or conceptualization of the study&lt;br&gt;Major role in the acquisition of data&lt;br&gt;Analysis or interpretation of the data&lt;br&gt;Drafting or revising the manuscript for intellectual content</td>
</tr>
</tbody>
</table>