Recent Advances in genetic and molecular markers of diffuse adult glioma; implication for diagnosis, prognosis and treatment

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Neuropathology services at Kings College Hospital
1. Brain tumours: Histology and IHC
2. Molecular genetics
3. Pituitary and base of skull tumours
4. Epilepsy pathology
5. Muscle pathology
6. Peripheral nerves and Small fibre neuropathy
7. Autopsy brain examination
   - Neurodegeneration- Brain Bank
   - Traumatic brain injury and sudden unexpected death
     (Forensic Neuropathology)
   - Epilepsy
   - Stroke and ischaemia
   - Inflammation and demyelination
   - Paediatric Neuropathology
Glioma WHO classification 2007

Astrocytic tumours
- Pilocytic astrocytoma (including pilomyxoid astrocytoma)
- Subependymal giant cell astrocytoma
- Pleomorphic xanthoastrocytoma
- Diffuse astrocytoma
- Anaplastic astrocytoma
- Glioblastoma

Oligodendroglial tumors
- Oligodendroglioma
- Anaplastic oligodendroglioma

Oligoastrocytic tumours
- Oligoastrocytoma
- Anaplastic oligoastrocytoma

Ependymoma
- Subependymoma
- Myxopapillary ependymoma
- Ependymoma
- Anaplastic ependymoma

Diffuse (infiltrative) Glioma
- Diffuse astrocytoma grade II
- Anaplastic astrocytoma grade III
- Glioblastoma grade IV (primary and secondary)
- Oligodendroglioma grade II
- Anaplastic oligodendroglioma grade III
- Oligoastrocytoma grade II
- Anaplastic oligoastrocytoma grade III
Incidence of Diffuse glioma, GBM, PA in South east London and Kent (Kings College Hospital)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>2002-11 total</th>
<th>2002-11 incidence per million per year</th>
<th>2012-15 total to Sept 2015</th>
<th>2012-15 incidence per million per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytoma, anaplastic</td>
<td>107</td>
<td>2.74</td>
<td>28</td>
<td>1.91</td>
</tr>
<tr>
<td>Astrocytoma, NOS</td>
<td>95</td>
<td>2.44</td>
<td>32</td>
<td>2.12</td>
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<tr>
<td>Astrocytoma, pilocytic</td>
<td>115</td>
<td>4.08</td>
<td>66</td>
<td>4.51</td>
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<tr>
<td>Glioblastoma</td>
<td>948</td>
<td>23.54</td>
<td>397</td>
<td>27.15</td>
</tr>
<tr>
<td>Glioma, NOS</td>
<td>58</td>
<td>1.49</td>
<td>34</td>
<td>2.32</td>
</tr>
<tr>
<td>Oligoastrocytoma, mixed</td>
<td>27</td>
<td>0.69</td>
<td>18</td>
<td>1.23</td>
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<tr>
<td>Oligodendroglioma, anaplastic</td>
<td>151</td>
<td>3.87</td>
<td>51</td>
<td>3.40</td>
</tr>
<tr>
<td>Oligodendroglioma, NOS</td>
<td>143</td>
<td>3.67</td>
<td>31</td>
<td>2.39</td>
</tr>
</tbody>
</table>

High and low grade glioma 54/million/y
- 35/Million/y Glioblastoma (GBM) grade IV
- 15/Million/y Diffuse glioma (DG): O, AO, A, AA, OA, AOA (II, III)
- 4/Million/y Pilocytic astrocytoma (PA) grade I

Problems in the diagnosis
Oligodendrogloma or astrocytoma?
Oligodendroglioma or astrocytoma

Astrocytoma II

Oligodendroglioma II
Problems in diagnosis of Low grade glioma

- Morphologically oligodendroglialoma II (OII) may look relatively straightforward diagnostically, the working neuropathologist are commonly encountering more ambiguous tumours with intermediate features between astrocytoma (AII) and oligodendroglialoma (OIII).
- Tissue sampling may not be complete
- There is a lack of consensus on the optimal diagnostic approach in these cases and particularly in defining oligodendroglialoma (OII) and oligoastrocytoma (OIII).
- The distinction between OII, OIII and AII is subjective and sometimes difficult
- Ueki et al 2002. 4 experienced neuropathologists reviewed 91 brain tumours
  General agreement 49/91 (54%)
  Disagreement on OII 71%, OIII 67%
- Does oligoastrocytoma truly represent a single definable clinico pathological entity or it simply represent morphological ambiguity in otherwise pure glioma?

What is Pilocytic astrocytoma?

There are many different histological types like:
- Oligodendroglialike cells similar to oligodendroglialoma
- Astrocytic component to similar to diffuse astrocytoma
- Vascular hyperplasia and even necrosis similar to GBM
- Occasional ganglion cells like cells similar to ganglioglioma
Is GBM different from other glioma
Molecular and genetic markers for diffuse adult glioma

- 1P/19q
- IDH1 and 2
- ATRX
- TERT
- BRAF
- EGFR vIII and EGFR amplification
- P53
- MGMT
P53 in glioma

1p/19q

Peri centric translocation of the 1p and 19q resulting in unbalanced formation with one copy of 1p and one pf 19q
1p/19q

Diagnosis
Frequent in oligodendrogloma (II, III)

Prognosis
• Associated with longer progression free survival
• Significant predictor to response to PCV and Temozolomid

Method
• Asses by FISH or LOH

Kings college Based evidence 2012
Relative frequencies of molecular markers in WHO types

1p and or 19q status

![Chart showing relative frequencies of molecular markers in WHO types](chart.png)
**Kings Based Evidence**

**Progression Free Survival (PFS) and Recurrence Free survival (RFS) for glioma with 1p/19q deletion**

**IDH1 and 2 (isocitrate dehydrogenase)**

- Mutation in IDH1, R123H (90%) and IDH2, R172K (10%)
  - reduce the isocitrate to 2 hydroxyglutrate “2HG” (instead of α-ketoglutarate)
  - accumulate to high level “HG” in glioma
- inhibit epigenetic modifier TET2 and hypermethylation of promotion of CPG
- glioma CPG island methylater phenotype (G-CIMP)
- global dysregulation of gene expression

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**R132H IDH1 mutation**

**R172K IDH2 mutation**
B Frequency of Mutations

A Glioblastoma

B Anaplastic Astrocytoma

Probability of Survival (%) vs Months

IDH wild-type

IDH mutated

P = 0.002

P = 0.001
IDH 1, 2

Diagnosis
- High frequency in diffuse glioma (DG) A II, AA III, O II, AO III, OA II, AOA III
- Mostly negative in primary Glioblastoma, pilocytic astrocytoma, ependymoma, neuronal and glioneuronal tumours
- Tumour marker for infiltrative tumour cells in a small or not representative biopsy

Prognosis
- Indicate better prognosis

Method
- IHC detect mutation in 90% (require gene sequencing in negative glioma II, III)

IDH1 positive in infiltrative glioma cell
ATRX

- ATRX (Alpha Thalassemia /mental Retardation syndrome X linked gene). Located on Xp21

- Essential part of lipoprotein complex regulating chromatin remodelling, nucleosome assembly, telomere maintenance and incorporation of Histone 3 into telomeric region of chromosome

- Loss of ATRX
  ↓
  Telomere dysfunction
  ↓
  Alternative lengthening telomere (ATL) phenotype

Diagnosis
- Common in astrocytoma, mainly in IDH +ve adult glioma
- Not seen with 1p/19q deletion (oligodendroglioma)

Prognosis
- Associated with better prognosis

Method
- IHC: Mutation cause loss of staining
- Difficult interpretation
- Positive in normal cells (neurons, inflammatory cells and endothelial cells)
- Heterogeneous staining but consider to be preserved if more than 10% of cells are positive
Telomerase transcriptase (TERT) mutation

- TERT encodes the enzyme that elongates telomeres and prevents cell degeneration from multiple rounds of mitosis
- Somatic mutation in C228T and C250T generate a new binding sites and increases TERT gene transcription
- Occurs in many human cancers including oligodendroglioma and GBM.
- Very rare in astrocytoma

TERT in Prognosis in glioma

TERT mutation indicate poor prognosis in GBM

TERT has no effect in the prognosis of LGG
EGFR amplification and EGFR VIII

EGFR amplification
50% of GBM
useful DD of GBM from others
  • Method FISH
EGFR vIII
  • 20-30% of primary GBM (associated with amplification)
  • May carry poor prognosis
  • Cell able to secrete membrane derived micro vesicles containing EGFR vIII mRNA and can be monitor for tumour response and relapse
  • Current trial based on therapeutic vaccination against the peptide

EGFR
BRAF fusion and mutation

BRAF point mutation – BRAF 600E

- 80% in Pleomorphic xanthoastrocytoma
- 50% ganglioglioma and glioneuronal tumour
- 5% in Pilocytic astrocytoma
- 2% in diffuse astrocytoma

Part of RAS/RAF signal pathway transmit extracellular signals from cytoplasm to nucleus

BRAF fusion

- BRAF fusion: truncated fusion mutation (KIAA1549-BRAF)
  ↓
  BRAF gene become under control of promoter of KIAA1549
  ↓
  Duplicate activation domain and deletion of N-terminal inhibitory domain

- Present 70-80% OF Pilocytic astrocytoma Absent in Other gliomas and glioneuronal tumour
KIAA1549-BRAF fusion by RT-PCR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mw 50 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>K16-9B</td>
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</tr>
<tr>
<td>K16-11B</td>
<td></td>
</tr>
<tr>
<td>K15-9B</td>
<td></td>
</tr>
</tbody>
</table>

KIAA1549-BRAF fusion in Pilocytic astrocytoma

MGMT Promoter methylation

**Function**
- Methyl Guanine Methyl Transferase repair the chemotherapy induced alkylation in the tumour thus counteract the effect of alkylating agent such as Temozolomide
- Hypermethylation of MGMT gene lead to silencing of the gene and reduce the protein levels
- Reduced level of MGMT protein increase the tumour ability to respond to treatment and improve patient outcome

**Prognosis**
- Methylated MGMT is associated with prolonged progression free survival in GBM compared with un methylated

**Method**
- Methylation specific PCR or pyrosequencing
Kings College Based Evidence

1p/19q co-deletion with IDH and ATRX mutations define the pathology and the prognosis of diffuse glioma better than the morphological classification

Ross Laxton and Safa Al-Sarraj
Clinical Neuropathology
Kings College Hospital
London
1p/19q co-deletion with IDH and ATRX mutations define the pathology and the prognosis of diffuse glioma better than the morphological classification

60 adults Low grade diffuse glioma LGG
- OII= 47
- All= 4
- OAll= 9

Prognosis
- No significant difference in recurrence free survival (PFS)
- Significant difference in progression free survival (0.03)

Estimated median survival
- All= 5.1 year
- OII, OAll= didn’t reach (long survival)

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Oligodendroglioma (n=47)</th>
<th>Oligoastrocytoma (n=4)</th>
<th>Atrctoma (n=9)</th>
<th>p=</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p/19q co-deletion</td>
<td>30 (64)</td>
<td>1 (25)</td>
<td>0 (00)</td>
<td>0.0005</td>
</tr>
<tr>
<td>IDH1 or IDH2 mutation</td>
<td>41 (91)</td>
<td>4 (100)</td>
<td>7 (88)</td>
<td>ns.</td>
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<tr>
<td>MGMT methylation</td>
<td>30 (73)</td>
<td>2 (50)</td>
<td>5 (63)</td>
<td>ns.</td>
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<tr>
<td>ATRX loss</td>
<td>11 (26)</td>
<td>2 (50)</td>
<td>5 (71)</td>
<td>0.03</td>
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<tr>
<td>P53 mutation</td>
<td>9 (27)</td>
<td>1 (25)</td>
<td>5 (63)</td>
<td>0.008</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (53)</td>
<td>1 (25)</td>
<td>6 (67)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22 (47)</td>
<td>3 (75)</td>
<td>3 (33)</td>
<td></td>
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<tr>
<td>Mean age years</td>
<td>43.4 (±13.6)</td>
<td>34.6 (±6.3)</td>
<td>43.1 (±13.7)</td>
<td>ns.</td>
</tr>
</tbody>
</table>

Results
- IDH 1,2 mutation in 91% in all low grade glioma
- 1p/19q co-deletion more in OII (64%)
- 1p/19q co-deletion is exclusive to ATRX mutation and p53
- IDH mutation in 100% of 1p/19q co-deletion (p= <0.05)
- ATRX mutation is more in All (71% and OAll (67%)
- P53 mutation is more in All (83%)
- Strong association between ATRX and p53 (p=0.0005)
Oligodendrogloma $n=47$

Oligoastrocytoma $n=9$

Astrocytoma $n=4$

**Group A $n=37$**

1p/19q co-deletion/IDH mutation
Molecular Oligodendroglioma

All have oligodendroglial differentiation
Very good prognosis

**Group B $n=16$**

ATRX loss/IDH mutation
Molecular astrocytoma

**Group C $n=5$**

IDH wt
Glioma IDH -ve

Majority are Astrocytoma
Intermediate prognosis

Very good prognosis

Majority are Astrocytoma
Intermediate prognosis

Poor prognosis
Probably precursors to GBM

![Graphs showing survival functions for IDH, 1p/19q, and ATRX](image)
Low grade diffuse glioma

Molecular classification

Histological classification

Molecular diagnosis

Histological diagnosis
Results
Risk of LGG progression
• Absence of IDH1,2
  IDH negative mutation (wild type) confers to 25 folds increased of risk of progression compared with IDH mutation
• Absence of 1p/19q co deletion
• PS3 mutation
• ATRX mutation
  ATRX mutation confers to 14 folds increase risk of progression compared with 1p/19q
  Singular 19q deletion
• Singular 19q deletion
  Shorter median RFS and PFS compared with the 1p/19 co deletion

<table>
<thead>
<tr>
<th></th>
<th>RFS</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td>Co-deletion (no)</td>
<td>4.4</td>
<td>1.89</td>
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<tr>
<td>IDH (WT)</td>
<td>4.7</td>
<td>1.56</td>
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<tr>
<td>MGMT (unmethyl)</td>
<td>2.1</td>
<td>0.90</td>
</tr>
<tr>
<td>PS3 (mut)</td>
<td>3.0</td>
<td>1.26</td>
</tr>
<tr>
<td>ATRX (loss)</td>
<td>1.8</td>
<td>0.82</td>
</tr>
<tr>
<td>K67</td>
<td>0.9</td>
<td>0.82</td>
</tr>
<tr>
<td>Age</td>
<td>1.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Gender (M)</td>
<td>1.0</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Molecular oligodendroglialoma
Molecular astrocytoma

ATRX negative

IDH1 positive

No 1p/19 deletion

Oligoastrocytoma or oligodendroglioma: 45 year old female patient with left frontal lobe tumour diagnosed as oligoastrocytoma

IDH1 positive

ATRX preserved

The end of Oligoastrocytoma
Molecular pilocytic astrocytoma

<table>
<thead>
<tr>
<th>Sample</th>
<th>K16-9B</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mw 50 bp</td>
<td>Negative control</td>
<td>Positive control</td>
<td></td>
</tr>
</tbody>
</table>

IDH1 negative

BRAF Fusion (70%)

Astrocytoma or Ganglioglioma?
19 years old female patient with left temporal lobe relatively well defined lesion diagnosed as astrocytoma grade II

IDH1 negative
ATRX preserved
Synaptophysin
**Oligodendroglioma or pilocytic astrocytoma**

21 year old patient presented with epileptic fit: CT sac: well defined tumour in the right occipital lobe with classification: diagnosed as oligodendroglioma WHO grade II.

- **ATRX preserved**
- **IDH1 negative**
- **BRAF fusion**

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**Primary and secondary GBM are different types of tumours**

- **Primary GBM (IDH wild type)**
  - No IDH mutation
  - No ATRX mutation
  - No 1p/19q deletion
  - Frequent TERT mutation
  - Frequent EGFR amplification

- **Secondary glioblastoma (IDH mutation)**
  1) Astrocytoma Grade IV
     - IDH1 mutation
     - ATRX mutation (lost)
     - No TERT mutation
     - No 1p/19q deletion
  2) Oligodendroglioma grade IV
     - IDH mutation
     - No ATRX mutation
     - TERT mutation frequent
     - 1p/19q deletion
Molecular **primary** GBM molecular profile

- IDH 1 negative
- ATRX preserved
- GFAP

**Secondary** GBM or anaplastic astrocytoma grade IV

- IDH1
- ATRX
- Ki67
Glioblastoma with oligodendroglial differentiation GBMO grade IV

Anaplastic oligodendroglioma with necrosis WHO grade III

IDH1 no mutation
ATRX no mutation
No 1p/19q deletion

IDH1 mutation positive
ATRX no mutation
1p/19q deletion

Introduction

Previously we have shown that glioblastomas with an oligodendroglial component (GBMO) did not differ from other glioblastomas in the frequency of 1p/19q deletion or isocitrate dehydrogenase (IDH) mutation.
Results

Hematoxylin and Eosin features

Oligodendrogloma
- 1p/19q co-deletion
- IDH1/2 positivity by immunohistochemistry or PCR
- ATRX preserved

Glioblastoma
- Absence of 1p/19q co-deletion
- Absence of IDH1/2

Classical anaplastic oligodendrogloma (AOIII) 30
Anaplastic oligodendrogloma with necrosis and endothelial cell proliferation (AOIII+) 16

Glioblastoma with oligodendroglial 46

Relative overall survival of AOIII vs. AO with necrosis vs. GBMO

Kaplan-Meier plot

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Median OS</th>
<th>Mean follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOIII</td>
<td>Not reached</td>
<td>3 years</td>
</tr>
<tr>
<td>AOIII + nec</td>
<td>66.5 months</td>
<td></td>
</tr>
<tr>
<td>GBMO</td>
<td>9 months</td>
<td>&gt; 1 year</td>
</tr>
</tbody>
</table>

- Significant differences in survival between all the groups
Oligodendroglioma n=47

Oligoastrocytoma n=9

Astrocytoma n=9

Group A n=37
1p/19q co-deletion/IDH mutation
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Group C n=5
IDH wt
Glioma IDH-ve
Poor prognosis
Probably precursors to GBM

30 11 4
7 2
3
1

The NEW ENGLAND JOURNAL of MEDICINE
Astrocytoma or low grade GBM precursor?

- MGMT unmethylated
- IDH1 negative

New WHO recommendation
Implications of integrated molecular and morphological diagnosis of gliomas

- The diagnosis becomes more accurate and less dependent on morphology and inter and intra observer variability
- End of Oligoastrocytoma: divided between oligodendroglioma and astrocytoma (depending on ATRX mutation and 1p/19q co-deletion)
- Primary (IDH wild) and secondary (IDH mutant) GBM are different types of tumour; The name of secondary GBM should be changed to anaplastic astrocytoma grade IV and anaplastic oligodendroglioma grade IV should be probably
- Wild type diffuse glioma have poor prognosis and probably represent precursor of GBM
- More related to actual prognosis of individual tumours
- Predictive factors for response to type of treatment
- Clinical trials become more specific for treatment on specific type of gliomas; therefore most likely to yield new and more effective treatments
- Targeted therapy according to the genetic alteration such as EGFR vIII and BRAF mutation

Consultation services histological and molecular diagnosis of brain tumours

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Kings College Hospital London

1910 King Edward VII laid the foundation of the third Kings College hospital at Denmark Hill

- 1828: Mr. Robert Bentley Todd obtained decision to establish KCH
- 8th October 1931 KCH opened its half-finished build (KCH) near Somerset House in the Strand
- 1881 the new building for the second KCH was opened

Kings College Hospital was officially opened in Denmark in 1913 by King George V and Queen Mary

- Now, kings is one of the largest hospitals in the UK and renowned for specialties in liver, haematology, renal and cardiac, stroke, neonatal medicine, diabetes and one of the largest Neurosciences centres in the UK.

College of Medicine, University of Baghdad

The first hospital opened 1927
Thank you

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