Is ALS a multistep process?

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Is ALS a multistep process?

- Multistage models
- Identification of stages
- Multistage model of ALS
- Implications for future research
Characteristics of cancer

- ( Mostly) adult onset condition
- Even those born with a genetic susceptibility will usually not get the disease till old age, or not at all
- The same gene mutation can result in different phenotypes
- Starts in one region (probably with just one cell) and then spreads
- Once it occurs, then progression is usually relentless
Models of Carcinogenesis: The Armitage-Doll Model

• Cancer is established in stages (probably 5 or 6)
• These stages must occur in a particular sequence
• These stages are irreversible
Normal cell → 1 → 2 → 3 → 4 → 5 → 6 → Cancer cell
The chance of $u_1$ occurring by age $t$ is $u_1 t$

The chance if $n-1$ steps occurring by age $t$ is $u_1 u_2 u_3 u_4 ... u_{n-1} t^{n-1}$

The chance of the last step then occurring is $u_n$
The incidence at age $t$ is approximately 

$$u_1u_2u_3u_4u_5u_6t^{n-1}$$

If the steps have to be in a particular order then this becomes:  

$$u_1u_2u_3u_4u_5u_6t^{n-1}/(n-1)!$$
Therefore at age t: \[ I = u_1 u_2 u_3 u_4 u_5 u_6 t^{n-1}/(n-1)! \]

\[ \log(I) = (n-1)\log(t) + c \] (where c is a constant)

If we assume that this needs to happen in at least one cell line (out of many) then we multiply by the number of cell lines (approximately);

The constant then changes, but the formula itself doesn’t
Therefore at age $t$:

$$\log(I) = (n-1)\log(t) + c$$  \hspace{1cm} (where $c$ is a constant)

This predicts a straight line relationship between $\log(I)$ and $\log(\text{age})$ with the slope being one less than the number of steps.
Stomach cancer incidence by age
Stomach cancer: $\ln(I)$ by $\ln(\text{age})$

$y = 5.0x - 17.8$
Some ‘limitations’ of the theory

- It works well for epithelial tumours, since epithelial cells have continual cell division throughout life.
- It works less well for tissues that have variable rates of division throughout life, e.g. childhood cancers, breast, neural, bone cancers.
- In the case of breast cancer, this is most likely because the theory does not directly allow for cell proliferation (e.g. as happens with breast tissue in puberty).
- In that situation, a factor can increase the risk of cancer simply by causing partially transformed cells to proliferate (thus making more copies which are susceptible to further changes).
Female breast cancer: ln(I) by ln(age)

\[ y = 3.9x - 10.0 \]
Male breast cancer: \( \ln(I) \) by \( \ln(\text{age}) \)

\[ y = 5.0x - 20.0 \]
Some testable implications of the theory

• The model predicts the ‘decline’ in acceleration at older ages (i.e. that the slope drops below that of a straight line)
• There are several ways that this can happen, but the most likely one is simply that for older people, they have some cells that have already gone through one or more stages
• What we see for each person then is a risk which is an average across cells, some of which have less than n stages to go
• The ‘average’ slope for a population is then less than n at older ages
Reduced acceleration at older ages: lung cancer incidence
Reduced acceleration at older ages: lung cancer acceleration
Some testable implications of the theory

• If a population is born with one step already ‘accomplished’ (e.g. if they are born with a genetic susceptibility) then their slope will be 1 less (e.g. if there are 6 steps, and they are born with 1, then they only have 5 steps to go and their slope will be 4)
Age-specific incidence of inherited and sporadic colon cancer.
Some testable implications of the theory

• If an exposure (e.g. smoking) acts at two stages, then incidence will be proportional to the square of the daily dose, and the \((n-1)\)th power of the number of years of exposure

• This is the case for smoking and lung cancer where the incidence is proportional to the square of the daily dose
Some testable implications of the theory

• Weighting exposure history can help identify likely stage(s) of action
South Carolina Asbestos Textile Worker Study
Exposure Time Windows

--- cumulative exposure ---

exposure time-window

person-year at risk
Exposure Time Windows

- 30+
- 25-
- 20-
- 15-
- 10-
- 5-
- 0-4
Exposure Time Windows

0 30+
1 25-
2 20-
3 15-
4 10-
5 5-
6 0-4

Unadjusted Adjusted
Models of Carcinogenesis

- cumulative exposure
- exposure time-window
- person-year at risk
Length of follow-up (f)

Duration of exposure (d)

Starts work (selection out)

Age at first exposure ($t_0$)

Age at increment of exposure ($t_0 + x$)

Age at end of exposure ($t_0 + d$)

Age at risk ($t$)

Stops work (selection out)
Under These Assumptions, the “Excess Risk” From Exposure at Age $T$ Is:

$$E(t) = \int_{0}^{d} \text{dose} \cdot (t_0 + x)^{j-1} (f-x)^{k-j-1} \, dx$$

$t_0 = \text{age at first exposure}$
$x = \text{time since first exposure at age } t$
$f = \text{length of follow-up at age } t$
$d = \text{duration of exposure}$
$k = \text{total number of stages}$
$j = \text{stage affected}$
Models of Carcinogenesis: The Armitage-Doll Model

• We do the analysis six times, each time assuming that asbestos acts solely at one of the six possible stages

• We then compare the six different models to see which best fits the data
## Models of Carcinogenesis: The Armitage-Doll Model

<table>
<thead>
<tr>
<th>Assumed stage</th>
<th>Categorical chi-square</th>
<th>Continuous RR</th>
<th>chi-square</th>
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</table>
Is ALS a multistep process?

• Multistage models
• Identification of stages
• Multistage model of ALS
• Implications for future research
What are the steps?

• ‘Background’ mutations rates are generally not large enough to explain the population incidence of epithelial tumours

• However, the population incidence rates are more ‘explainable’ if some steps involve:
  – Mutation to the DNA repair system
  – Epigenetic and chromosomal changes
  – Physiological changes
  – Clonal expansion
  – Increases in the mutation rate
What are the steps?

There are few instances where all or most of the stages have been established. These vary greater between and within cancers, but in general:

• The early stages often involve somatic mutations or chromosomal aberrations
Example: colorectal cancer

1. Mutation of the APC regulatory pathway (also occurs in small benign tumors)
2. Mutation of a RAS gene
3. Loss of parts of chromosome 18q
4. Loss of functional p53 (loss of protective control over cellular birth and death)

[50-85% of colorectal cancer follow this path]
Example: colorectal cancer

1. Inherited mutations in mismatch repair system (MMR)
2. Microsatellite instability
3. Mutation to \(APC\) or \(\beta\)-catenin
4. Mutations in \(K-RAS\), \(TGF\beta-RII\)

[2-4% of colorectal cancers follow this path]
Is ALS a multistep process?

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Characteristics of ALS

• Adult onset condition
• Even those born with a genetic susceptibility will usually not get the disease till old age, or not at all
• The same gene mutation can result in different phenotypes (e.g. same mutation can predispose to ALS/FTD/ALS-FTD, but also to schizophrenia, depression and Parkinson's disease)
• Starts in one region (perhaps with just one cell) and then spreads
• Once it occurs, then progression is usually relentless
• Cancer and neurodegeneration are very different phenomena: cancer is an uncontrolled proliferation of cells, whereas neurodegeneration is the result of the death of cells. Nevertheless, they share several key characteristics
Parallels with cancer

- Multiple hits affect a small group of cells
  - Spread from this small group
  - Relentless progression

- Anatomy of spread determined by mechanism of spread
  - Metastasis vs invasion
A simple model of ALS risk

Health

Burden of disease causing factors

No disease

Disease

Al-Chalabi and Hardiman, Nature Reviews Neurol 2013
ALS might be a multistep process

- ALS requires independent steps in order
  - $u$ is a small probability
  - $t$ is age
  - Each step occurs at rate $u$ per year
  - Probability of any step after $t$ years is $ut$
  - At age $t$ probability that $n$ steps have occurred is $(ut)^n$
  - Last step has probability $u$
  - Incidence = $u(ut)^n$

- $\ln(\text{incidence}) \sim n\ln(\text{age})$
- Disease requires $n+1$ steps
SEALS Register

Netherlands Register

Irish Register

Scottish Register

<table>
<thead>
<tr>
<th>ALS Register</th>
<th>Sex</th>
<th>Slope (n)</th>
<th>95% CI Lower limit</th>
<th>95% CI Upper limit</th>
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<td><strong>Total</strong></td>
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</tbody>
</table>
How many steps to cause ALS?

Adapted from Armitage and Doll, Int J Epidemiol 2004 by Neil Pearce, LSHTM UK

\[ y = 4.8x - 18.1 \]

Suggests 6 steps needed
Do we see the same pattern for MS?
Subgroup analyses

Population subgroup analyses are difficult:

• For certain risk factors (e.g. head injury) we do not have population denominator data and cannot calculate incidence rates for the population subgroups with these characteristics.

• For other factors (e.g. smoking) we can (roughly) estimate population denominators, but we would still not expect to see clear patterns because these are risk factors which may cause a step to occur in some people – they are not markers of a step.

• Other factors (e.g. genetic mutations) may directly correspond to a step, and prevalence is (more or less) constant across age-groups, so we can compare ‘incidence rates (using the total population for denominators) between those who have and don’t have the factor.
Subgroup analyses

Case subgroup analyses are more straightforward:

• All case subgroups have the same (total population) denominators when calculating incidence rates
• Thus, we can compare the age-specific incidence rates (and slopes) for different types of cases
Conclusions

• All ALS has a major genetic component

• Multistep model explains many features of ALS

• ALS subgroups exist
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Implications for future research

The multistep model is inconsistent with:

• The hypothesis that the pathological process is present from birth, but that the toxic effect of the disease-causing protein takes time to build up to sufficient levels to trigger disease

• The hypothesis that ALS involves a dose-dependent, two-step process of genetic risk and subsequent environmental triggers
Multistage model of ALS is consistent with observation

- Neuronal specificity
  - Vulnerability can be subgroup specific
- Genetic pleiotropy
  - The gene variant is only one of several steps
- Spread from a focus
  - Only some neurons will have had all necessary hits
- Age dependent incidence
  - It takes time to accumulate all hits
- Genetic and environmental risk
  - Hits in multiple domains
- Reduced genetic penetrance
  - Individuals not exposed to all steps will remain well
Implications for future research

The multistep model is consistent with:

• The abiotrophy hypothesis which posits that a toxic insult specifically depletes motor neurons: the toxic insult would be one of the steps

• A recently proposed model of neurodegenerative diseases being the result of somatic mutations.

• The hypothesis that ALS is the result of long-term aggregation of protein or seeding of prion-like domains in cellular proteins: one of the steps could include protein aggregation in a subset of cells; a further step could include failure to clear the toxic aggregates in a smaller subset; the site of onset can be explained because only some neurons will have undergone all steps
Implications for future research

• Individuals with a mutation that accounts for one step might be expected to have a log(age) versus log(incidence) slope of 4 rather than 5
• Environmental exposures are probably important for all types of ALS
• ALS may still be preventable in those with genetic susceptibility
• Different exposures may cause the same stage to occur
• Pleiotropy may be important – different neurodegenerative diseases may have common causes
Implications for future research

- Identification of stages
- Use of multistage model to identify aetiologically distinct subgroups
- Is ALS the same disease in different parts of the world?
- Analyses of other neurological conditions
Patients and families
Is ALS a multistep process?

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