THE CHANGING PICTURE OF AMYOTROPHIC LATERAL SCLEROSIS: LESSONS FROM EUROPEAN REGISTERS

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Title: 86 characters

Word count abstract:	102
Word count article:	3590
Tables:	2
Figures:	3

References: 59

Keywords: Epidemiology; disease register; population based; prospective

Author contributions:

OH conceived the study. OH & JR wrote the first draft.

AAC, CB, EB & LvdB, AC, SM, GL, provided expert opinion and contributed to subsequent drafts.

SM and JR prepared the tables and figures.

All authors approved the final manuscript.

Funding Statement:

The research leading to these results has received funding from the Health Research Board Interdisciplinary Capacity Enhancement Programme, the European Community's Seventh Framework Programme (FP7/2007-2013) under the Health Cooperation programme and the project EUROMOTOR (number 259867), from the European Joint Programme in Neurodegeneration (SOPHIA and ALS-CarE), and the Charities Research Motor Neuron and Irish Motor Neuron Disease Association. The funding sources played no role in the preparation of this manuscript.

Disclosures and conflicts of interest

Orla Hardiman is funded by the Health Research Board Clinician Scientist Programme. Prof. Hardiman has received speaking honoraria from Novarits, Biogen Idec, Sanofi Aventis and Merck-Serono. She has been a member of advisory panels for Biogen Idec, Allergen, Ono Pharmaceuticals, Novartis, Cytokinetics and Sanofi Aventis. She serves as Editor-in-Chief of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia.

Ammar Al-Chalabi declares associations with OrionPharma, Cytokinetics, Mitsubishi-Tanabe Pharma, OneWorld Publications and Cold Spring Harbor Laboratory Press.

Carol Brayne declares no conflicts of interest.

E. Beghi received personal compensation from GSK and UCB-Pharma for speaking at an epilepsy congress, and from Viropharma for being part of a steering committee. Dr Beghi received personal compensation from Epilepsia as Associate Editor. Dr Beghi received research support from UCB-Pharma for studies in epilepsy and Parkinson's disease, and from GSK for studies in epilepsy.

L.H. van den Berg serves on scientific advisory boards for the Prinses Beatrix Spierfonds, Thierry Latran Foundation, Biogen and Cytokinetics; received an educational grant from Baxalta; serves on the editorial board of Amyotrophic Lateral Sclerosis And Frontotemporal Degeneration and the Journal of Neurology, Neurosurgery, and Psychiatry; and receives research support from the Prinses Beatrix Spierfonds, Netherlands ALS Foundation, The European Community's Health Seventh Framework Programme (grant agreement n° 259867), The Netherlands Organization for Health Research and Development (Vici Scheme, JPND (SOPHIA, STRENGTH, ALSCare)).

Adriano Chio serves on the editorial advisory board of the journal 'Amyotrophic Lateral Sclerosis and Frontotemporal Dementia' and has been a member of advisory panels Biogen Idec, Cytokinetics, Italfarmaco and Neuraltus.

Sarah Martin is funded by the MND Association, the National Institutes for Health Research Biomedical

Research Council at South London and Maudsley NHS Foundation Trust and King's College London, and the Collaboration for Leadership in Applied Health Research and Care South London.

Giancarlo Logroscino, serves as associate editor for the Neuroepidemiology Journal, Karger. He received founding from Ministry of Health. Ministry of University and Research, <<regione Puglia Special program for research.

James Rooney is funded by the Health Research Programme Clinical Fellowship Programme.

Acknowledgements:

The authors would like to thank Dr Katy Tobin who kindly prepared Figure. 2

Abstract

Prospective population based Registers of amyotrophic lateral sclerosis (ALS) have operated in Europe for over two decades, and have provided important insights into our understanding of ALS. Here we review the benefits that population Registers have brought to the understanding of the incidence, prevalence, phenotype and genetics of ALS, and, outline the core operating principles that underlie these Registers and facilitate international collaboration. Going forward we offer lessons learned from our collective experience of operating ALS registers in Europe for over two decades.

Introduction

Prospectively designed population based registers with complete ascertainment of all affected individuals in a defined geographic catchment area are of increasing value in epidemiologic research, as evidenced by the success of European population-based registers for Amyotrophic Lateral Sclerosis (ALS)[1,2].

Although a rare disease with an incidence in Europe of 2-3/100,000 person-years[1,2], ALS provides an excellent model to study neurodegenerative diseases. The disease has a rapid progression and is uniformly fatal, and there is high clinico-pathological correlation (autopsy diagnosis matches clinical diagnosis), rendering in vivo diagnosis accurate in the vast majority of cases. As is the case with other neurodegenerations, it is now recognized that ALS is a heterogeneous condition associated with more than one pathogenic mechanism and with different clinical manifestations and trajectories. With evolving recognition of disease heterogeneity as an important factor in a precision medicine approach towards the development of new therapeutics, population based datasets can play a crucial role in defining the full range of disease phenotype and demography.

ALS population based Registers capture all cases regardless of age, health or socioeconomic status, and can thus provide a wealth of information about disease incidence, prevalence, spatial distribution, heterogeneity in clinical phenotype, outcome and analysis of risk. The Irish and Italian ALS Registers have been in continuous operation for over 2 decades, and the Dutch and English for over a decade, during which insights into the patho-physiology, epidemiology and genetics of ALS have developed greatly. Each of these registers has provided valuable country-specific data regarding epidemiology, disease progression, clinical phenotyping and genetics, health service planning, quality of life assessment and mapping of the patient journey[2–7].

The EURALS consortium in Europe, which combines ALS population based Registers from European countries, has generated a simplified framework for inclusion in an ALS database including basic demographics, El Escorial categorization at diagnosis, site of onset, time from first symptom to onset, cognitive status using a standardised screening tool with country specific validation, and genotype if known[8]. This has enabled collaboration across European Registers, providing insights that would not be

otherwise possible. For example, combined appraisal of Registers has indicated the presence of population based variability, has provided evidence that ALS is a multistep process[5], and has facilitated unbiased studies of spatial epidemiology[9–11].

The aim of this review is to provide a detailed analysis of what has been learned from existing ALS Registers, to identify and to recognize hidden biases and confounders which may affect Registers, and to explore future challenges to population based Registers and how these might be met.

Incidence and Prevalence of ALS

Comparative analysis of ALS across European Registers has provided incidence rates of 2.6/100,000 person-years and prevalence rates of 7-9/100,000 persons, with a mean life expectancy of 30 months from first symptoms[2,12,13]. European ALS Registers are well served by the presence of stable populations with defined geographic borders ensuring accuracy of incident and prevalent figures [2,14]. All patients are captured by population based registers because no patient is too old, too poor or too sick. A stable population structure with limited mobility also reduces the risks of loss to follow up. Population based Registers differ in this respect from Registers that are based on service utilization, drug prescription or insurance cover, as these are likely to miss patients who do not attend specialists or access services, and repositories are accordingly biased.

In general, multiple data sources provide the best mechanism for accurate data capture[1,14]**.** Depending on the type of health system, European ALS Registers have ascertained patients by a combination of unique patient identifiers (UK, Italy), referrals through networks of clinical professionals and death certification (Ireland), self-reporting and coding, and face to face or telephone based interviews with self-reported questionnaires (Netherlands). To assess the degree of under-ascertainment when two or more independent sources are used, a capture-recapture system is often used [13,15], although this is less accurate if data sources are linked in any way as has been shown by the Irish Register[16].

How hidden bias can affect estimates of incidence

A number of studies have suggested that the incidence of ALS may be increasing[17–19]. However, careful evaluation of European Register data collected over 20 years suggest otherwise[14]. Thus, these apparent increases in incidence are most likely to be explained by the increased recognition of phenotypes that might in the past have been excluded from collection, including in some patients with "possible ALS" as per the EL Escorial diagnostic criteria and those with features of fronto-temporal dementia (FTD), who also have a recently recognized associated motor degeneration. Slight changes in accepted criteria for inclusion on Registers in turn leads to subtle shifts in ascertainment, as Registers evolve to include patients who might otherwise have escaped ascertainment, particularly in later life[20]. The apparent increase in cases is contemporaneous with the evolving recognition that ALS patients can experience a range of cognitive changes which in some cases may be the presenting symptom[21,22]. Additionally, all Registers, no matter how well they are designed, will miss some cases at the beginning. The development of a national Register, or indeed the presence of a specialist service within a particular region, can have a secondary effect of increasing awareness of the condition, which also improves ascertainment as time goes on. Thirdly, the demographics of European countries are such that an increasing proportion of the population has entered the age range associated with increased risk of developing ALS. In this instance, although the total number of cases of ALS might increase within a population, the standardized rates may not have changed.

Failure to recognize the inherent biases of subtle changes in disease recognition, coupled with improvements in ascertainment strategies and changes in population demography can drive assumptions about increasing frequencies of ALS that are not fully supported by appropriately adjusted data.

How biases in datasets can affect survival estimates

Population based Registers have demonstrated that up to 70% of patients with ALS die within 3 years of first symptoms, and that approximately 5-10% can survive for more than 8 years[12,23]. Some clinical trial-based datasets (e.g. ProACT (https://nctu.partners.org/ProACT)) have suggested that survival of ALS patients has increased over time, and has been attributed in part to increased use of interventions such as

non-invasive ventilation. However, while there is evidence that non-invasive ventilation may affect survival in some patients, data from European population based Registers has not identified a significant overall effect on survival at population level since the inception of NIV as a standard of care in ALS management[12,23]. Moreover, detailed analysis of population based registers suggests that the apparent increase in survival described in some clinical trial based datasets is more likely to be a function of systematic. European population based Registers have demonstrated that those patients who participate in clinical trials, regardless of whether the therapeutic agent has been efficacious, have a different disease trajectory compared to the disease trajectory of the overall population based cohort[24]. Table 1 and figure 1 exemplify this by comparing participants of the **Lithium in patients with amyotrophic lateral sclerosis** (LiCALS) trial to the parent register population, i.e. the South-East England ALS (SEALS) register[25]. This is for the reason that trial datasets usually comprise prevalent patients who attend specialist clinics and who are sufficiently well to meet trial entry criteria and sufficiently motivated to enroll in clinical trials. It has been long recognized that differences in disease trajectories between incident and prevalent cohorts can bias survival analyses, and that this bias underpins the differences in clinical characteristics between clinic-based (primarily prevalent) and population-based (incident and prevalent) cohorts [26]. It is therefore more likely that improvements in survival in data repositories such as ProACT are due to subtle differences in the composition of disease cohorts rather than a true shift in disease behaviour. However, it is also the case that direct comparison of datasets (including Registers), across different epochs as a means of determining changes in disease outcome can lead to unintentional bias due to cohort effects. This is one of the reasons why the use of historical controls for comparative purposes is therefore not recommended, even when using population based data from Registers. This is because most European ALS Registers have shown that data captured in the early years of Registers is unlikely to be of the same complexity or quality of subsequently captured data. For example, in the Irish ALS Register, a subtle shift in age profile can been ascertained within the study cohort [20]. This is most likely to reflect a transition in awareness of disease within the elderly population among referring practitioners. Maturing Registers eventually shift detection from a mixture of prevalent and incidence cases in the earlier Register years towards ascertainment of

incident cases as the Register became established (Figure 2). Comparative analyses within Registers and across different periods must therefore take these potential confounders into account in drawing conclusions about disease behaviour.

Expanding the ALS Phenotype

Italian and Irish ALS Registers provide detailed analyses of disease sub-phenotypes that have helped to characterize the clinical and cognitive changes associated with ALS [27–30], demonstrating how Registers provide new insights that can help to accurately classify patients into different clinical and prognostic subgroups. This can be helpful for clinical trial stratification. For example, Irish and Italian Registers have shown that cognitive and behavioural changes are intrinsic features of ALS, affecting up to 50% of patients[28], and are associated with significant prognostic implications [29,30]. Interrogation of individual datasets can also provide important differentiating features that allow exclusion of possible mimic syndromes [31].

Registers have also been helpful in characterizing the presence of possible endo-phenotypes, among probands and their extended family members. Such population-based observations can in turn lead to novel and previously unrecognized pathogenic mechanisms. For example, a recent population based study from Ireland has shown higher rates of psychosis and suicide in first and second degree relatives of ALS patients compared to controls [32]. This observed family aggregation of neuropsychiatric disorders in ALS kindred's provided the necessary hypothesis to undertake a combined summary statistics GWAS Analysis of ALS and schizophrenia which has revealed a hitherto unrecognized 14% polygenic overlap between ALS and schizophrenia, suggesting the presence of shared pathogenic mechanisms between these two clinically divergent disorders [32].

Ascertaining Environmental Risk

While Registers of themselves, cannot define risk, well established population based prospective Registers such as the Dutch ALS Register can support detailed population based case control studies aimed to assess

environmental risk, including physical activity, BMI, consumption of alcohol and fat, smoking and other exposures [6,33–35]. Because ALS is a rare disease, large case control studies require extensive collaboration between different centres and across different geographic regions. Sublte differences in ascertainment and disease definition can introduce bias unless the data collection has been standardized with respect to ascertainment and characterization. The recent Euro-MOTOR study comprising cases and controls from countries with population based Registers demonstrates that combined ascertainment can significantly enhance power for risk assessment[36]. The Euro-MOTOR project has now established a repository of over 1500 population based incident cases and 3000 matched controls with extensive phenotype, environmental, and genomic characterization[36]. The Euro-MOTOR design has since been exported to other regions, most recently to 3 Latin American countries (Cuba, Chile and Uruguay) which have formed the Latin American Epidemiology Network for ALS (LAENALS).

Registers can also demonstrate how non-uniformity of access to health systems can bias analyses of risk. This is exemplified by conflicting observations relating to the association of disease risk and socioeconomic status. For example, an association between ALS with area-based socio-economic status was reported in New Jersey [37], where access to health services is not uniform and those in lower socioeconomic groups may not be captured. This contrasts with recent analyses from the European Registers, which have not demonstrated any association between social deprivation and ALS incidence [9–11].

Registers can also be used to address claims of disease clustering. Many studies have been published suggesting increased rates of ALS in regions thought to be associated with specific risk. This is exemplified by the suggestion that environmental pollutants or cyanotoxin exposure are associated with ALS. A geographic ascertainment bias (the so called “Texas sharp-shooter phenomenon”) is generated by examination of reported clusters in the absence of complete surveillance data [38]. By contrast careful population based analysis using well established Registers has to date failed to identify a spatial association between specific environmental pollutants and disease risk, as exemplified most recently by the negative evidence for clustering in a heavily polluted region of Italy[39]. Indeed, to date other than Guam and the Kii peninsula of Japan, no reproducible areas of clustering have been noted. A region of reduced incidence

("cold spot") has recently been reported by the Irish Register [40]. The reasons for this are unclear, but may be related to subtle historical differences in local population structure.

Making Sense of Genetics

Defining Familial Disease

Prior to the recognition of the importance of the C9orf72 repeat expansion as an important causative gene in ALS, familial ALS (FALS) has been reported to account for 5% of cases [41]. More detailed analysis of family history and genotyping of at least one population based Register (Ireland) now suggests that the true proportion of FALS is closer to 16-20% of all ALS cases [32]. Low reported rates of familial disease are most likely a function of biased study design that do not collect within a population-based setting. It is also the case that incident patients may not be aware of a family history, or may not recognize the link between the proband and a family history of progressive neurological decline. Longer running Registers can provide important insights in this regard, as new patients are ascertained within kindred's that had previously been classified as "sporadic".

It must also be noted that genetic studies that do not recognize the presence of variations in population structure can confound analysis, as the make assumptions of uniform prevalence of gene variants and clinical phenotype [42,43]. Variation in the prevalence of at risk genes is known to be the case for at least two major ALS genes - the frequency of the C9orf72 repeat expansion is high in populations of European extraction and low in the Asian population [44], while variants in SOD1 account for 13% of familial ALS in Italy, but are not found in Ireland and are rare in Holland [45]. The presence of population isolates can also affect the genetic epidemiology of ALS. For example, higher rates of ALS have been identified in a population isolate in The Netherlands, leading to the discovery of ALS associated variants in the NEK1 gene [3]. Similarly, higher rates of familial ALS have been noted in Sardinia due to founder effects with respect to TDP43 and C9orf72[43].

It is important to note that Registers can not only help to identify new genes as in the case of NEK1, they can also limit the impact of referral bias on genetic studies [46]. Many genetics studies are of necessity clinic based, and since it is known that clinic based cohorts are phenotypically distinct from population based cohorts (Table 1), it can be assumed that reports of the prevalence of at risk genotypes are also biased. It should also be noted that apparently minor differences in selection processes (e.g. clinic rather than population based patients, and controls that are not matched) have the potential to influence the outcome of genetic association studies.

Complex Genetics, Ancestral Origin and Disease Risk

Interrogation of incidence, prevalence and risk genetically admixed populations is of increasing interest. As noted, there is now considerable evidence that the incidence of ALS varies significantly across countries [47,48] and the phenotype and outcome of the disease vary in relation to population ancestral origin [49]. South American populations of mixed ancestral origin may have lower rates of ALS compared with those reported in Europe [47,50]. A population based mortality study from Cuba has reported different rates of ALS in different ancestral populations, with higher rates in those of European origin and lower rates in the admixed population (which corresponds to the "Latino" population in the US) [47]. Population based Registers that ascertain within a region of mixed ancestral origin are therefore of particular interest from genetic and environmental perspectives. Differences in ancestral risk may be significant sources of bias in the generation of Registers in countries such as the US, where differential access to and utilization of health services is linked to race, ethnicity, language, rurality, and socioeconomic status [51,52]. The design of European population based ALS Registers can counteract such biases by capturing all patients using multiple different sources and care pathways [2].

Health Services

Population based Registers can inform health services. The availability of precise incident, prevalent and clinical trajectory data can permit detailed service planning, and can enable projection of future societal

needs. While Registers cannot of themselves provide sufficiently rich datasets to inform the entire patient journay, well constructed and compatible Registers within different jurisdictions can also permit comparative analyses of different types of services, as has been demonstrated in the island of Ireland [53]. Comparison of survival outcome between Registers in the Republic of Ireland and Northern Ireland, which have similar population structures but which provide different types of specialist care for ALS patients, have shown the multidisciplinary care within a single clinic is superior to devolved care provided within a defined "hub and spoke" model of care [53]. Registers also permit high level comparative studies of different interventions within individual geographic regions using outcomes such as hospitalizations and survival. In Puglia, the model of care is such that there is no survival difference between patients attending local neurologists and those receiving care in a specialist multidisciplinary clinic [54]. Similarly, nested work by Dutch researchers shows that the additional availability of a regional care worker does not improve quality of life among carers [55], although analysis of outcome using population based datasets in the Netherlands and Lombardy (Italy) show that multidisciplinary clinics are also better value for money, reduce hospitalizations and enhance quality of life of patients [56,57].

Health Services, Clinical Trials and "Real World Data"

There is an increasing recognition that clinical trials by necessity select patients that are not representative of the true population, rendering decisions regarding the generalization of trial findings challenging from a health policy perspective [58]. While the best study design for assessment of treatment effectiveness is the randomized clinical trial, therapeutic effectiveness can also be assessed in more generalizable context using prospective observational studies nested within Registers, as has been demonstrated in the case of Riluzole[59]. Prospective cohort studies using population based Registers in which the outcomes are collected after exposure, or intervention in patients can provide valuable "real world" information regarding the longer term effect of a therapeutic intervention.

Sustainability of Population Based Registers.

Registers are difficult to fund as they are often viewed as infrastructure by research bodies, and as research initiatives by health services. Many Registers rely on the energy and interest of a single founder, and are challenged at the time of retirement of the key principal investigator, as occurred in the case of the Scottish Register on the retirement of the founder clinician, Dr. Robert Swingler. Fortunately, recent recognition of the value of the population-based Register for ALS by the Scottish health authorities has enabled the re-establishment of this valuable resource with the provision of ring fenced funding.

Long term sustainability of Registers can also be eroded by limitations on the types of data disease Registers are permitted to record. While issues of privacy and data protection must be clearly addressed in Registers as part of an over-arching governance structure, recent changes in European legislation are of potential concern to the operation of true population based Registers. For example, inclusion of data relating to living patients without their expressed informed consent is now in breach of European data protection laws. As institutional review boards are taking an increasingly stringent position regarding patient's autonomy with the introduction of "consent to contact" requirements, there is now a real risk of under-ascertainment of cases. Without derogation based on the principles of public health benefit, it becomes increasingly difficult to create and sustain accurate population based Registers for most conditions.

Legislation providing derogation will require an understanding and recognition by the public of the important potential societal benefits of population-based epidemiological research, and in particular the potential public health benefit of identifying and communicating data regarding regional variations in disease incidence, prevalence and survival. This principle is implicit in the case of notifiable infectious and communicable diseases and is of particular import in the case of rare neurodegenerative. This has been recognized in some jurisdictions for some types of Registers (e.g. the Irish Cancer Register, and the US ALS Registry), and by the International Rare Disease Research Consortium (http://www.irdirc.org/). However, there remains a disappointingly limited recognition within the Europe legislature of the significant benefits of population based Registers.

Conclusions

Prospective population-based disease registers are invaluable in patient oriented research of rare diseases. As exemplified by the success of European ALS Registers, population based databases can identify and address biases that are intrinsic to other types of data. While some biases cannot be completely eliminated, their recognition can provide the necessary caution in data interpretation. Notwithstanding their limitations, Registers can provide unique and often unexpected insights into disease epidemiology and pathobiology, and can inform the types of healthcare that are of greatest benefit to patients.

It is imperative that funding agencies, healthcare providers and institution review bodies recognize the value of these types of Registers, particularly in the case of rare disease such as ALS, and that forthcoming data protection legislation, while well intentioned and appropriate in many ways, does not compromise our ability to fully understand disease heterogeneity, and to continue to improve the lives of patients with ALS and related neurodegenerations.

Core Recommendations

- Clearly defined case definitions should be used including inclusion and exclusion criteria.
- Include a clearly labeled register subsection for cases that should be tracked but do not fulfill the formal inclusion criteria.
- Register variables should be carefully selected and a “core content” paradigm should be agreed in advance.
- International collaborative efforts and/or national merger of data in large countries using multiple registers to cover different regions are advisable for rare diseases.
- Dedicated staff time to ensure effective set-up and maintenance of the register
- Defined Capture methodology including multiple sources
- Regular comparison of ascertainment rates and patient demographics
- Investigation of “ascertainment holes”
- Employment of careful statistical analyses of data collected in the first 3-5 years to account for *“start-up bias”*
- Exclude the most recent 1-2 years of data capture, particularly for survival analyses.
- Security is paramount for system software, yet flexibility to accommodate a shifting knowledge base is essential.
- Including population-based controls in a register enables valuable case-control studies for studying environment/lifestyle/genetic risk factors

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Table 1. Demographic and clinical characteristics of patients enrolled in the LiCALS trial versus the SEALS population based register

Variable		**SEALS (n=296)**	**LiCALS (n=217)**	**Comparison (test)**
Mean age at onset (years)		63.5	58.0	**p-value <0.001** (t test)
Median diagnostic delay (months)		12.5	9.8	**p-value <0.001** (Mann-Whitney U)
Sex (M:F %)		52:48	70:30	**p-value < 0.001** (Chi squared)
Site of onset (%)	Bulbar	29.3	21.7	**p-value = 0.004** (Chi squared)
	Spinal	66.9	78.3	
	Not recorded	3.7	0	
El Escorial (%)	Definite	27.7	38.2	**p-value <0.001** (Chi squared)
	Probable	48.6	37.3	
	Clinically probable-laboratory supported	1.7	18	
	Possible	11.8	6.5	
	Suspected	2.7	0	
	Not recorded	7.4	0	

Table 1 Legend: Comparison of patients enrolled in the **Lithium in patients with amyotrophic lateral sclerosis** (LiCALS) study[25] compared to the population based South-East England ALS (SEALS) register from which participants were recruited.

Table 2. Abridged ENCALS – ALS core clinical dataset

Personal data	Clinical Data	Genetics	ALSFRS	ECAS Cognitive and Behavioural data
Date of birth	Diagnosis	Which ALS genes tested ?	Date of ALS-FRS	ECAS additional clinical data
Date of death	Date of diagnosis	Mutation in ALS gene ?	ALS-FRS 12 subscores	ECAS cognitive data
Gender	Co-morbidities	Free text notes	ALS-FRS total scores	ECAS behavioural data and carer interview
Patient/control	El Escorial category	Free text to add other genes	Rater name	
ID numbers	Date of disease onset			
Optional identifying data	Site of disease onset			
Free text notes	Other first symptoms (e.g. weight loss, cognitive/behavioural)			
	Forced Vital Capacity / Sniff nasal inspiratory pressure			
	Family history			
	Endpoints – date of death, date of tracheostomy, date of			

	NIV > 23hrs/day, date of latest endpoint check			

See full ENCALS ALS core clinical data guidelines on the ENCALS website[8]

Figure 1. Kaplan-Meier curve of LiCALS vs SEALS data

Figure 1 Legend: Kaplan-Meier survival curves for patients enrolled in the LiCALS trial (blue) versus the population based SEALS register from which participants were recruited. Characteristics of these groups are described in Table 1.

Figure 2 Proportion of illness prior to diagnosis versus year of death

Figure 2 Legend: The above graph shows time for onset to diagnosis as a proportion of time from onset to death by year of death for incident cases on the Irish ALS register from 1995 - 2013. In the early years of the register this proportion appears higher before stabilizing to a more consistent ratio after the year 2000. This could be representative of improving survival during the early years of the register however this would be at odds with our previous survival analysis [13]. Alternatively, this could be explained by the detection of 'legacy' prevalent cases being detected in the early years of a population disease register. Note that this graph is generated only from cases diagnosed from 1995 and onwards. If this trend is explained by pre-existing 'legacy' cases in the population, who may have developed the disease some years previously, the effect represents a 'start-up bias' in the early years of the disease register, as such cases are by definition likely to be long survivors.