

Identification of natural TSC-Associated Neuropsychiatric Disorders (TAND) clusters

By

Loren Leclezio

MSc (Med) Neuroscience, PhD Candidate

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Health, Faculty of Health Sciences,

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Supervisor

Professor Petrus J de Vries, University of Cape Town

Statistical collaborator

Professor Sugnet Gardner-Lubbe, University of Stellenbosch

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I, Loren Leclezio, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisor and contributions from others as outlined in the acknowledgements. The assistance I received with data collection, analysis and manuscript review from the co-authors of the publications that form part of this thesis is described for each relevant chapter.

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1. Leclezio, L. and de Vries, P.J. (2015). **Advances in the treatment of tuberous sclerosis complex.** *Current Opinion in Psychiatry.* **28**, pp. 113-120
2. Leclezio, L., and de Vries, P.J. (2016). **Towards an improved understanding of TSC-associated neuropsychiatric disorders (TAND).** *Advances in Autism.* **2**, pp. 76-83
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Signed

SIGNATURE:

DATE: 15 August 2017

STUDENT NAME: Loren Leclezio

STUDENT NUMBER: LCLLOR001

CONTENTS

| | |
|---|-----------|
| List of Tables..... | 6 |
| List of Figures..... | 7 |
| Abstract..... | 8 |
| Acknowledgements | 10 |
| Abbreviations..... | 11 |
| Key Terms | 12 |
| | |
| Chapter 1: Introduction | 13 |
| 1.1 Background to the thesis | 13 |
| 1.2 Background to Tuberous Sclerosis Complex | 14 |
| 1.3 The Neuropsychiatric Phenotype of TSC | 24 |
| 1.4 Aims of the Thesis | 25 |
| 1.5 Author contributions to included manuscripts | 26 |
| | |
| Chapter 2: Advances in the treatment of Tuberous Sclerosis Complex | 27 |
| 2.1 Introduction | 27 |
| 2.2 International TSC Consensus guidelines and recommendations | 28 |
| 2.3 TSC-Associated Neuropsychiatric Disorders (TAND) | 31 |
| 2.4 The TAND Checklist | 36 |
| 2.5 The hope of molecularly targeted treatments for the neuropsychiatric manifestations of TSC | 37 |
| 2.6 Key Points | 39 |

| | |
|---|-----------|
| 2.7 Conclusion | 39 |
| 2.8 Chapter Summary | 40 |
| | |
| Chapter 3: Towards an improved understanding of Tuberous Sclerosis Associated Neuropsychiatric Disorders (TAND) | 41 |
| 3.1 Introduction | 41 |
| 3.2 The Neuropsychiatric Phenotype of TSC | 42 |
| 3.3 The Assessment and Management Gap of TSC | 45 |
| 3.4 Perceived Uniqueness of TAND Profiles | 46 |
| 3.5 Treatment Paralysis in TAND | 48 |
| 3.6 Potential next steps to improving our understanding of TAND | 48 |
| 3.7 Conclusion | 51 |
| 3.8 Chapter Summary | 51 |
| | |
| Chapter 4: Identification of natural clusters of TSC-Associated Neuropsychiatric Disorders - A Pilot feasibility study | 53 |
| 4.1 Introduction | 53 |
| 4.2 Methods | 56 |
| 4.3 Results | 58 |
| 4.4 Discussion | 62 |
| 4.5 Conclusion | 64 |
| 4.6 Chapter Summary | 64 |

| | |
|---|------------|
| Chapter 5: Multivariate data analysis identifies natural TAND clusters | 66 |
| 5.1 Introduction | 66 |
| 5.2 Methods | 67 |
| 5.3 Results | 71 |
| 5.4 Discussion | 78 |
| 5.5 Conclusion | 83 |
| 5.6 Chapter Summary | 83 |
| | |
| Chapter 6: Conclusions | 85 |
| 6.1 Introduction | 85 |
| 6.2 Thesis Overview and Conclusions | 86 |
| 6.3 Implications of this Research | 86 |
| 6.4 Limitations of this Study | 88 |
| 6.5 Future Directions | 89 |
| | |
| References | 91 |
| | |
| Appendix A: TAND Checklist | 106 |
| Appendix B: Ethical approval HREC | 110 |
| Appendix C: Informed Consent | 111 |
| Appendix D: Chapter 4 supporting material | 116 |
| Appendix E: Chapter 5 supporting material | 120 |

LIST OF TABLES

Chapter 1

| | |
|--|----|
| Table 1.1: Clinical trials of TSC-Associated Neuropsychiatric Disorders | 22 |
|--|----|

Chapter 2

| | |
|--|----|
| Table 2.1: Updated diagnostic criteria for tuberous sclerosis complex | 29 |
|--|----|

| | |
|---|----|
| Table 2.2: TSC-Associated Neuropsychiatric Disorders | 32 |
|---|----|

Chapter 3

| | |
|---|----|
| Table 3.1: Multiple levels of TSC-Associated Neuropsychiatric Disorders (TAND) and reported rates | 42 |
|---|----|

Chapter 4

| | |
|--|----|
| Table 4.1: TAND Checklist items included in the pilot study | 54 |
|--|----|

| | |
|---|----|
| Table 4.2: Exploratory factor analysis results | 60 |
|---|----|

Chapter 5

| | |
|---|----|
| Table 5.1: Bootstrapping results | 74 |
|---|----|

| | |
|---|----|
| Table 5.2: Factor analysis results identifying a seven-factor solution | 75 |
|---|----|

| | |
|--|----|
| Table 5.3: Chronbach's alpha scores for the seven natural TAND clusters Identified through WARD's cluster analysis | 76 |
|--|----|

| | |
|---|----|
| Table 5.4: Chronbach's alpha scores for the seven TAND factors identified through exploratory factor analysis | 76 |
|---|----|

| | |
|--|----|
| Table 5.5: Proposed seven natural TAND clusters with items contained in each cluster, and Chronbach's alpha scores per cluster | 81 |
|--|----|

LIST OF FIGURES

Chapter 1

- Figure 1.1:** Intracellular signaling and the neurobiology of tuberous sclerosis complex (TSC)15
- Figure 1.2:** Physical manifestations observed in TSC18
- Figure 1.3:** Age-dependant expression of clinical manifestation in TSC21

Chapter 3

- Figure 3.1:** The variability of TSC-Associated Neuropsychiatric Disorders (TAND)47

Chapter 4

- Figure 4.1:** Hierarchical cluster analysis with WARD's methods produced six clusters58
- Figure 4.2:** WARD's cluster analysis results in a heatmap59
- Figure 4.3:** Comparison of WARD's cluster analysis and exploratory factor Analysis61

Chapter 5

- Figure 5.1:** Diagram illustrating steps followed during statistical analysis.....68
- Figure 5.2:** Dendrogram illustrating WARD's cluster analysis results72
- Figure 5.3:** Comparison of WARD's cluster analysis and exploratory factor Analysis77

ABSTRACT

Tuberous Sclerosis Complex (TSC) is associated with many learning, behavioural, neurodevelopmental and psychiatric difficulties. Over 90% of individuals with TSC will have some of these concerns yet no more than 20% receive support and treatment, even though these issues may cause the greatest burden of disease in TSC. The Neuropsychiatry Panel at the 2012 TSC Consensus Conference coined the term TAND (TSC-Associated Neuropsychiatric Disorders) to capture the multidimensional concerns seen in TSC, and recommended that each person with TSC should be screened for TAND every year. To facilitate the process, a TAND Checklist was designed. Many professionals and families feel overwhelmed by the complexity of TAND and say that they do not know where to start and how to access relevant information, tips or 'next step' approaches. This may in part be due to the multi-dimensionality of TAND, and in part due to lack of access to clear, useful and evidence-based resources for TAND. This project aimed to examine the complexity of TAND. The hypothesis was that, even though each individual will typically have their own unique TAND profile, there will be key natural TAND Clusters – combinations of behaviours across multi-dimensional levels - that will simplify further evaluations and treatment. The study was performed over 36 months, in two phases using a mixed-methods approach. **Phase I** was a pilot phase. TAND Checklist data were collected from 56 individuals with TSC in South Africa (n=20) and in Australia (n=36). Using R, these data were explored with various multivariate data analysis techniques to identify suitable analysis methods for the identification of potential natural TAND clusters. WARD's cluster analysis method rendered six TAND clusters with good face validity, and convergence with a six-factor exploratory factor analysis solution. Pilot results suggested that a combination of cluster analysis and exploratory factor analysis methods may be able to identify clinically-meaningful natural TAND clusters. **Phase II** set out to replicate and expand on pilot results. TAND checklist data were collected from n=453 across six international TSC sites, and the multivariate analysis techniques identified in phase I were applied. WARD's method rendered seven natural TAND clusters with good clinical face validity and good statistical robustness on bootstrapping.

Results showed significant convergence with an exploratory factor analysis solution. Combining all data-driven strategies, we identified a 'Scholastic' cluster of TAND manifestations, a 'Neuropsychological' cluster, a 'Mood/Anxiety' cluster, an 'ASD-like' cluster, a 'Dysregulated Behaviour' cluster, a 'Overactive/Impulsive' cluster, and an 'Eat/Sleep' cluster. All natural clusters, apart from the Eat/Sleep cluster showed good to excellent internal consistency. The larger-scale study findings were remarkably consistent with pilot findings, supporting the robustness of these naturally occurring clusters. We propose that the seven natural TAND clusters identified can in future be used to generate educational and clinical resources for use in real-life settings. In addition, findings suggest that the aetiology and molecular treatments of TAND may also show differential clustering across human and animal models, pointing towards novel hypotheses regarding neuropsychiatric phenomena in TSC to be explored in future studies.

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Finally, I would like to thank and acknowledge the Lord in this process, Amen.

STYLE, ABBREVIATION, AND KEY TERMS

A note on spelling and style convention: UK English spelling has been used throughout this thesis. I have used Harvard referencing throughout the thesis for the same reason, and compiled all references at the end of this thesis.

ABBREVIATION

| | |
|-------------|--|
| ADHD | Attention deficit hyperactivity disorder |
| AMPK | AMP-activated protein kinase |
| ASD | Autism spectrum disorder |
| MAPK | Mitogen-activated protein kinases |
| mTOR | Mammalian target of rapamycin |
| TAND | Tuberous sclerosis associated neuropsychiatric disorders |
| TSC | Tuberous sclerosis complex |

KEY TERMS

Attention deficit hyperactivity disorder (ADHD)

Autism spectrum disorder (ASD)

Behavioural phenotype

Cluster analysis

Factor Analysis

Natural TAND clusters

Neuropsychiatric disorders

Tuberous sclerosis associated neuropsychiatric disorders (TAND)

Tuberous sclerosis complex (TSC)

Chapter 1

INTRODUCTION

1.1 Background to the thesis

This thesis has a primary focus on TSC-Associated Neuropsychiatric Disorders (TAND) and on a search for potential natural TAND clusters. The thesis was drafted to incorporate a number of peer-reviewed publications (with appropriate authorisation from the Doctoral Degree Board of the Faculty Health Sciences at the University of Cape Town). Given the multi-system nature of Tuberous Sclerosis Complex (TSC), the thesis will start with some general background to TSC, incorporating basic descriptions of the disorder and its history, the genetic and molecular aspects of the condition, and a topline introduction to TSC-associated neuropsychiatric disorders (TAND). The introductory chapter will be followed by two further background chapters. Chapter 2 will present a recent update of advances in the field of TSC, will summarise the revised diagnostic criteria for TSC, and will comment broadly on molecular treatments for TSC. The core of chapter 2 will focus on TAND and development of the TAND Checklist, given the focus of this thesis. Chapter 3 represents a conceptual reflection on TAND and on the reasons why individuals with TAND around the globe receive so little assessment and intervention. This chapter therefore provides the scientific rationale for the data-based work, which is presented in chapters 4 and 5. In chapter 4, a pilot feasibility study is presented. The pilot study was conducted with the aims of testing various multivariate data analysis techniques and to ascertain the feasibility of identifying natural TAND clusters. The findings from this pilot study formed the foundation for the large-scale study comprising 453 individuals with TSC. In chapter 5 of the thesis this study is presented and multivariate data analysis techniques identified in the pilot study applied. Chapter 6 concludes the thesis and summarises potential next steps and limitations of the work.

1.2 Background to Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder with multi-system involvement. In 1880 French physician, Bourneville, was first to coin the term “tuberous sclerosis of the cerebral convolutions” to describe the potato-like lesions seen during the autopsy of a girl who died as a result of seizures (Bourneville, 1880). TSC can affect virtually any organ system, with most commonly encountered physical manifestations being benign tumours in the central nervous system, skin, kidneys, heart, and lungs. TSC is also associated with a vast range of neuropsychiatric disorders that include behavioural problems, neurodevelopmental disorders, and major psychiatric disorders.

The disorder is caused by mutations in either of two genes, the *TSC1* gene on chromosome 9q34 (Povey *et al.*, 1994; van Slegtenhorst *et al.*, 1997) or the *TSC2* gene on chromosome 16p13.3. (The European Chromosome 16 Consortium 1993; Povey *et al.*, 1994). The encoded protein products of *TSC1* (hamartin) and *TSC2* (tuberin) regulate and integrate intracellular signalling pathways (**Figure 1.1**), including the PI3K, AMPK and MAPK pathways (de Vries and Howe, 2007; van Slegtenhorst *et al.*, 1997). A mutation in either of the TSC genes may disrupt the TSC1-TSC2 intracellular protein complex, resulting in hyperactivation of the mammalian target of rapamycin (mTOR) pathway. TSC is therefore an mTOR overactivation syndrome. The TSC-mTOR pathway is a signalling pathway that critically regulates cell growth and proliferation, protein synthesis, and metabolism (Kenerson *et al.*, 2002; Tee *et al.*, 2002). Developments in the molecular biology of TSC identified that the TSC-mTOR and related signaling pathways are also involved with numerous neurobiological processes including long-term potentiation (LTP) and synaptic plasticity, myelination, actin cytoskeletal formation, forebrain development, energy sensing, astrocyte morphology, and neuronal polarity, contributing to the neuropsychiatric phenotypes observed in TSC (summarised in Ehninger *et al.*, 2008; Costa-Mattioli *et al.*, 2009; de Vries, 2010b).

Serfontein and colleagues (2010) studied the evolution of the TSC1/2-TOR signalling pathway using complete genome sequencing and found that the existing pathway was built up from an ancestral one linking growth and energy

supply. The authors proposed that, after the divergence of the main eukaryote lineages, the pathway became more sophisticated in some lineages through the incorporation of additional input (such as TSC1-TSC2) and output elements (Serfontein *et al.*, 2010).

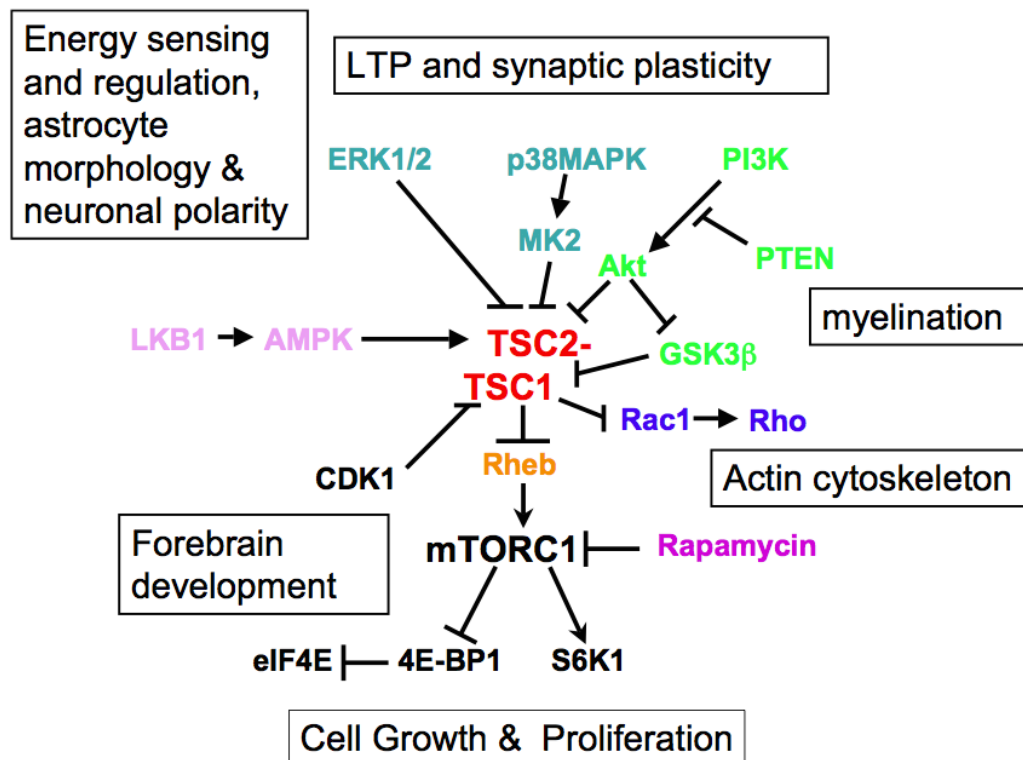


Figure 1.1. Intracellular signaling and the neurobiology of tuberous sclerosis complex (TSC). The TSC1-TSC2 protein complex acts as an intracellular complex at the crossroads of a number of important signaling pathways. These include the insulin-signaling PI3K-AKT pathway; the mitogen-activated p38MAPK and ERK1/2 pathways; and the energy-sensing AMPK pathway. Proteins in these pathways have fundamental neurobiological roles as represented schematically in the figure. 4E-BP1 = eukaryotic initiation factor 4E binding protein 1; AKT = protein kinase B; AMPK = adenosine monophosphate activated protein kinase; CDK1 = cyclin-dependent kinase 1; EIF4E = eukaryotic translation initiation factor 4E; ERK1/2 = extracellular signal regulated kinase 1 and 2; GSK3beta = glycogen synthase kinase 3 beta; LKB1 = serine/threonine kinase 11; LTP = long-term potentiation; MK2 = MAPK-activated protein kinase 2; mTORC1 = mammalian target of rapamycin complex 1; p38MAPK = p38 mitogen-activated protein kinase; PI3K =phosphoinositide 3 kinase; PTEN = phosphatase and tensin homolog; Rac1 = Ras-related C3 botulinum toxin substrate 1; Rheb = Ras homologue enriched in brain; Rho = Ras homologue gene family, member A; S6K1 = ribosomal p70 S6 kinase 1; TSC1 = tuberous sclerosis complex 1 protein (hamartin); TSC2 = tuberous sclerosis complex 2 protein (tuberin). Reproduced with permission from de Vries, 2010b.

The estimated birth incidence of TSC is 1 in 5,800 (Osborne *et al.*, 1991; O'Callaghan *et al.*, 1998). TSC appears to have an equal male/female distribution and is equally common in all ethnic groups and all countries. Two thirds (~70%) of cases occur as spontaneous mutations and the remaining ~30% are familial, inherited in autosomal dominant fashion (Jones *et al.*, 1997). Routine diagnostic techniques indicate that a pathogenic mutation is detected in up to 85-90% of individuals with a clinical diagnosis of TSC and the remaining ~ 10% with 'no mutation identified' (Nellist *et al.*, 2015; Tyburczy *et al.*, 2015). It seems likely that mosaicism accounts for a significant fraction of those without an identified mutation (Kwiatkowski *et al.*, 2015). There is significant phenotypic variability in the amount and severity of physical features of the disorder (summarised in Povey *et al.*, 1994; Curatolo *et al.*, 2008; Henske, *et al.*, 2016). Appropriate management and coordination of medical specialist care is vital across the lifespan to limit morbidity and mortality in individuals with TSC (Krueger *et al.*, 2013).

In 1967, Lagos and Gomez who were based at the Mayo Clinic, reported findings from a family of 71 individuals (over five generations) with TSC. These data led to the first set of diagnostic criteria. The so-called 'Gomez' criteria were revised in 1979 (Gomez, 1979) and again in 1998 following the first TSC consensus conference. The aim of this conference was to develop consensus recommendations for diagnosis (Roach *et al.*, 1998) and clinical management of individuals with TSC (Roach *et al.*, 1999). The last ~20 years have seen a number of momentous advances in understanding the pathogenesis of TSC and available treatment options: In the 1990s, the *TSC1* and *TSC2* genes were discovered (The European Chromosome 16 Consortium 1993; and Van Slechtenhorst *et al.*, 1997) and in the early 2000s, a molecular diagnostic test for TSC was launched. In 2001, the *Drosophila* homologues, *Tsc1* and *Tsc2*, were found to be involved in cell and organ size regulation (Potter *et al.*, 2001; Tapon *et al.*, 2001). It was further found in 2002 that tuberlin (TSC2 protein) is a target of the PI3K/AKT signalling pathway (Potter *et al.*, 2001; Tapon *et al.*, 2001; Dan *et al.*, 2002; Manning *et al.*, 2002) and that the TSC1 and TSC2 proteins form an intracellular complex (Tee *et al.*, 2002). These advancements led to discovering the critical role of the TSC genes in regulation of the mTOR pathway (Kenerson *et al.*, 2002).

Thereafter, rapamycin, an mTOR inhibitor, was shown to reduce renal tumours in Eker rats (Kenerson *et al.*, 2005) and mouse models (Lee *et al.*, 2005), and to reduce the size of SEGAs (Franz *et al.*, 2006) and renal angiomyolipomas in individuals with TSC (Bissler *et al.*, 2008; Davies *et al.*, 2008). There has been a number of large-scale clinical trials of mTOR inhibitors for peripheral organ systems involved in TSC, and more recently, investigating the effects of mTOR inhibitors on neuropsychiatric manifestations seen in TSC (summarised in Curatolo *et al.*, 2015; Henske *et al.*, 2016; ClinicalTrials.gov).

Given these key advances in TSC, a second international TSC consensus conference was held in 2012 bringing together 79 individuals (with various areas of expertise) from 14 countries (Northrup *et al.*, 2013; Krueger *et al.*, 2013). The aim of which was to revisit and reach consensus on diagnostic criteria, surveillance, and treatment guidelines for individuals with TSC.

A variety of organs are involved in TSC and manifestations can occur at various times during an individual's lifespan (See **Figure 1.2** for physical manifestations observed in TSC). One of the organ systems most commonly affected is the brain, with manifestations seen in 90-95% of individuals with TSC. The main neuropathological features of TSC are cortical dysplasias which include cortical tubers (CT) and white matter radial migration lines, subependymal nodules (SENs), and subependymal giant cell astrocytomas (SEGAs) (Franz *et al.*, 2010; Kingswood *et al.*, 2017). SENs typically occur in the area of the foramen of Monro in the lateral wall of the lateral ventricle and usually remain static throughout an individual's lifetime. Yet, in at least one-fifth of individuals with TSC progressive growth of a SEN lead to SEGAs. SEGAs typically occur within the first 20 years of life (Franz *et al.*, 2010). They generally exceed 1cm in diameter (but can grow to >10cm), causing hydrocephalus, focal neurological deficits, and even death (Goh *et al.*, 2004). These three lesions (CT, SEN, SEGAs) demonstrate shared histopathological features including abnormal cellular morphology, different regional architecture, and excessive numbers of astrocytes (Crino *et al.*, 2010). For growing but otherwise asymptomatic SEGAs, either surgical resection or medical therapy with mTOR inhibitors can be effective treatment options (Krueger *et al.*, 2010; Franz *et al.*, 2013; Northrup *et al.*, 2013).

Skin Manifestations



Hypomelanotic macule (white patch)



Shagreen patch



Ungual fibroma



Facial angiofibromas

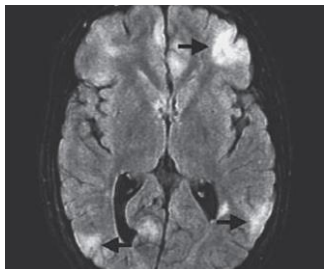


Facial angiofibromas

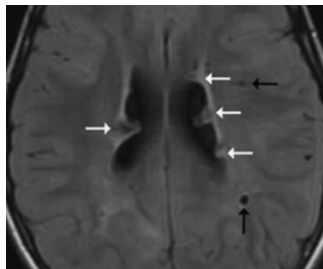


Fibrous cephalic plaques

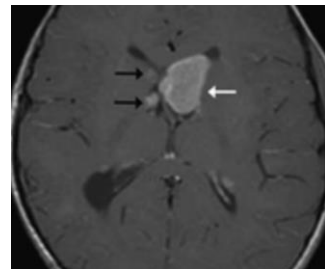
Brain Manifestations



Cortical tubers (black arrows)

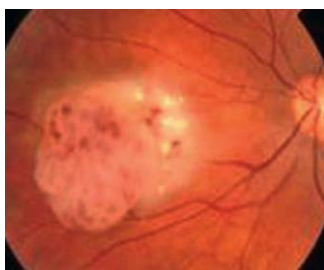


Subependymal nodules
(white arrows)



Subependymal giant cell
astrocytoma (white arrow)

Other Manifestations



Retinal hamartoma



Bilateral renal angiomyolipomas



Lymphangiomyomatosis (LAM)

Figure 1.2. Physical manifestations observed in TSC. From the top these show various skin and brain manifestations, as well as retinal hamartoma, renal angiomyolipomas, and lymphangiomyomatosis. Reproduced with permission from de Vries *et al.* (2017). Images kindly provided by Dr Chris Kingswood and Dr Raj Newaj.

Epilepsy is the most common neurological disorder in TSC, affecting 70-90% of individuals (Webb *et al.*, 1991; Thiele, 2004; Kingswood *et al.*, 2017). Seizure onset occurs within the first year of life in a significant proportion of individuals. Partial and partial complex seizures are common in TSC, as well as secondarily generalised seizures. A third of infants with TSC will develop infantile spasms and a proportion will develop Lennox-Gastaut syndrome (LGS), both potentially catastrophic epilepsy syndromes (Muzykewics *et al.*, 2007; Kingswood *et al.*, 2017). Anticonvulsant medications are currently used as first-line treatment options for seizures. However, 60-75% of individuals with TSC who have seizures will develop refractory epilepsy not effectively controlled by medical therapy (Kwan and Brodie, 2000; Chu-Shore *et al.*, 2010). Epilepsy surgery can be an effective therapeutic option, especially for children with TSC who have refractory epilepsy. Other treatment strategies for refractory epilepsy may include ketogenic diet and vagal nerve stimulation. More recently, early phase results have suggested that mTOR inhibitors may be an anti-epilepsy treatment in TSC. After a promising phase II trial (Krueger *et al.*, 2010), a multi-centre, international trial was initiated to evaluate the efficacy and safety of mTOR inhibitors as adjunctive treatment for partial seizures in TSC (French *et al.*, 2016; ClinicalTrials.gov: NCT01713946 (EXIST-3)). This study showed Everolimus (mTOR inhibitor) to have good efficacy as adjunctive treatment for treatment-resistant focal seizures. Everolimus has recently received marketing authorisation for this indication by the European Medicines Agency (http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002311/human_med_001484.jsp&mid=WC0b01ac058001d124).

The second most frequently affected organ system in TSC is the skin, with manifestations also seen in 90-95% of individuals with TSC (Teng *et al.*, 2014). TSC skin lesions have a characteristic appearance and include hypomelanotic macules (oval shaped off-white spots also known as ‘ash leaf spots’), facial angiofibromas (small reddish spots or bumps that consist of fibrous tissue and blood vessels), unguis fibromas, shagreen patches (firm irregular plaque with coalescing papules and nodules) and “confetti” skin lesion (small hypopigmented macules) (Northrup *et al.*, 2013). Even though these skin lesions are benign, they

may be painful and lead to bleeding. They can compromise normal functions of the nasal passage or nails, and distorts normal skin structures (Darling *et al.*, 2010).

The kidney is affected in 80-85% of individuals with TSC across their lifespan and include renal cysts and angiomyolipomas (AML) (Bissler *et al.*, 2013; Kingswood *et al.*, 2017). AMLs are benign tumours composed of vascular, smooth muscle, and adipose tissue. Hemorrhage from renal AMLs (proportional to size) can cause serious issues and lead to need for dialysis, renal transplantation, and sometimes mortality in adults with TSC (Bissler and Kingswood, 2004; Patel *et al.*, 2005; Bissler *et al.*, 2013).

Ophthalmological features include retinal hamartomas and are seen in ~50% of individuals with TSC (Thiele and Jozwiak, 2010; Kingswood *et al.*, 2017). These lesions have similar histological features to the brain tubers seen in TSC, are generally benign and typically do not affect vision (Northrup *et al.*, 2013).

Cardiac rhabdomyomas are the earliest detectable hamartoma, often seen on foetal ultrasound or foetal MRI, and are the only lesions in TSC likely to regress with age (Jozwiak and Respondek-Liberska, 2010). These tumours are believed to occur in 50% of individuals with TSC (summarised in Jozwiak and Respondek-Liberska, 2010; Kingswood *et al.*, 2017) and can be associated with cardiac arrhythmias including atrial and ventricular arrhythmia and Wolff-Parkinson-White syndrome (O'Callaghan *et al.*, 1998).

Lymphangiomyomatosis (LAM) is the primary pulmonary manifestation of TSC and occurs predominantly in females (Costello *et al.*, 2000; Franz *et al.*, 2001; Kingswood *et al.*, 2017). Cystic pulmonary parenchymal changes consistent with LAM are observed in 30-40% of female patients with TSC, although these women are frequently asymptomatic (summarised in McCormack and Henske, 2010; Northrup *et al.*, 2013; Kingswood *et al.*, 2017). LAM is characterised by a proliferation of smooth muscle cells in nodules throughout the lung and associated with interstitial expansion of the lung (Northrup *et al.*, 2013).

TSC has a clear age-related expression pattern (see **Figure 1.3**), with some

features more prevalent during infancy and childhood and others more likely to manifest during adolescence or in adulthood (summarised in Crino *et al.*, 2006; Curatolo *et al.*, 2008; Henske *et al.*, 2016).

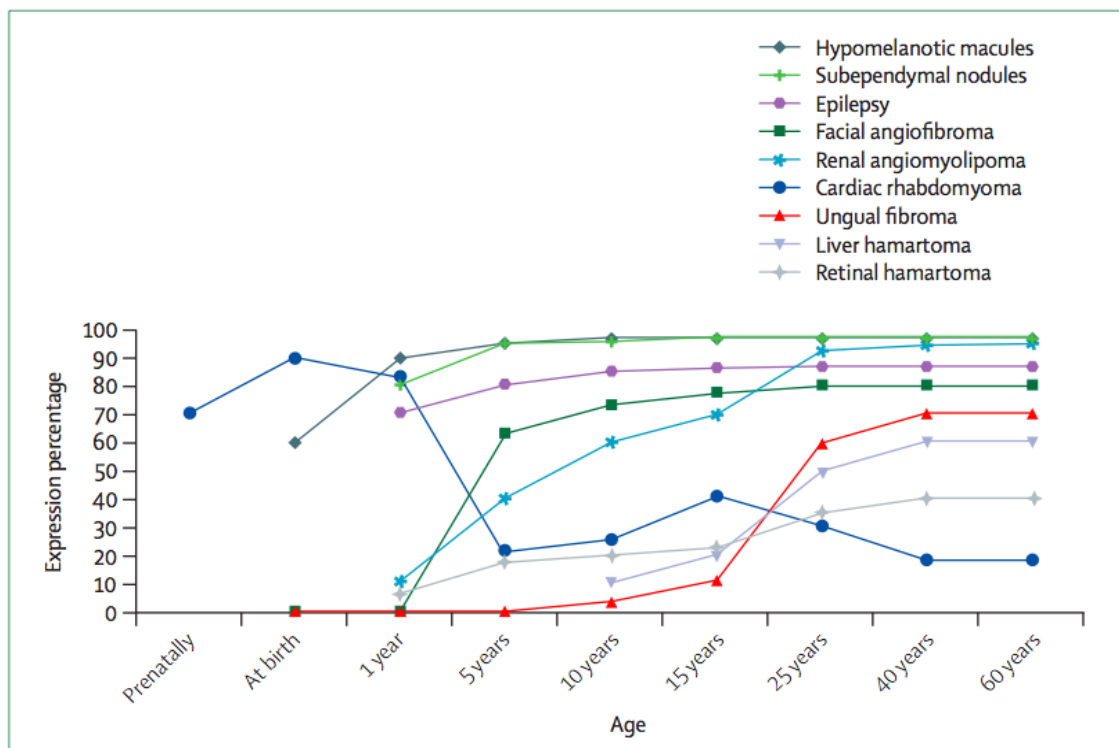


Figure 1.3. Age dependant expression of clinical manifestation in TSC. Reproduced with permission from Curatolo, P., Bombardieri, R., and Jozwiak, S. (2008).

At present no known cure exists for Tuberous Sclerosis Complex. Nevertheless, an understanding of the functional relationship between the TSC1-2 complex and mTOR has led to significant clinical advances in the use of mTOR inhibitors. Everolimus (Votubia®, Afinitor®) has been licenced by the European Medicines Agency (EMA) and Federal Drug Administration (FDA) for the treatment of SEGA not amenable to surgery, for angiomyolipomas >3cm, and, in Europe, for the adjunctive treatment of focal seizures. There are ongoing studies of mTOR inhibitors for the treatment of various physical manifestations of TSC including SEGA, AML, skin, LAM and epilepsy (NCT00789828; NCT00790400; Krueger *et al.*, 2013). There are also several trials underway studying the efficacy and safety of mTOR inhibitors across neurological and neuropsychiatric manifestations. See **Table 1.1** for clinical trials underway registered on ClinicalTrials.gov.

Table 1.1 Summary of key interventional neuropsychiatric and clinical trials in Tuberous Sclerosis, reproduced with permission from Curatolo *et al.*, 2015

| | | | | | | |
|---|-----------------------|---------------------------------|---|---|-----------|----------------|
| <p>Trial of RAD001 and neurocognition in tuberous sclerosis complex (TSC) (NCT01289912)</p> | <p>Interventional</p> | <p>Everolimus vs. placebo</p> | <p>To assess the safety and efficacy of everolimus in children and young adults with tuberous sclerosis. Outcome measures included standardised neuropsychological instruments</p> | <p>To identify signs of change in epilepsy, sleep, autism spectrum characteristics, academic skills, and behavioural difficulties</p> | <p>50</p> | <p>Phase 2</p> |
| <p>TRON (NCT01954693)</p> | <p>Interventional</p> | <p>Everolimus vs. placebo</p> | <p>To assess the efficacy of everolimus in the treatment of neuropsychological deficits. Outcome variables include episodic memory, planning, spatial working memory, and dual tasking</p> | <p>To identify additional neuropsychiatric markers of change. Measures include psychiatric questionnaires, quality of life, adaptive behaviours, and autism rating scales</p> | <p>48</p> | <p>Phase 2</p> |
| <p>RAPT (NCT01929642)</p> | <p>Interventional</p> | <p>Everolimus and Sirolimus</p> | <p>To assess the feasibility and safety of mTOR inhibitors in children with tuberous sclerosis and self-injurious behaviour. Outcome measures include compliance, carer burden, and parental Stress</p> | <p>To measure behavioural change (including self-injury, repetitive behaviours, and autism characteristics) and EEG and MRI changes</p> | <p>3</p> | <p>Phase 2</p> |

| | | | | | | |
|------------------------|----------------|---------------------------|---|--|----|--------------|
| RAPIT (NCT01730209) | Interventional | Everolimus vs. placebo | To assess the efficacy of everolimus in the treatment of autism and neuropsychological deficits in children with intellectual disability. Primary outcome is intelligence quotient as measured by the Wechsler Preschool and Primary Scale of Intelligence | To assess efficacy on other neuropsychiatric characteristics. Outcome variables of interest include autism, working memory and attention, sleep, sensory profiles, and epilepsy | 60 | Phase 2/3 |
|------------------------|----------------|---------------------------|---|--|----|--------------|

1.3 The Neuropsychiatric Phenotype of TSC

Individuals with TSC could present with a variable range of neuropsychiatric challenges across multiple levels (de Vries, 2010a; de Vries *et al.*, 2015a). These range from behavioural difficulties, psychiatric disorders, intellectual disability, academic/scholastic disorders, to neuropsychological deficits and psycho-social difficulties. A more detailed review of the neuropsychiatric phenotype is presented in Chapter 2.

Over 90% of individuals with TSC will have some of these concerns yet no more than 20% receive support and treatment (de Vries *et al.* 2015a), even though these issues may cause the greatest burden of disease in TSC. Even more recent data support this view (Kingswood *et al.*, 2017). The Neuropsychiatry Panel at the 2012 TSC Consensus Conference coined the term “TAND” (TSC-Associated Neuropsychiatric Disorders) to capture the multidimensional concerns seen in TSC, and advocated that each person with TSC should be assessed for TAND every year (Krueger *et al.*, 2013; de Vries *et al.*, 2015a). In order to facilitate the screening process, a TAND Checklist was developed for use as a tool to guide healthcare teams in a systematic investigation across the levels of neuropsychiatric investigation seen in TSC (de Vries *et al.*, 2015a; Leclezio *et al.*, 2015). These will be discussed in more detail in chapter 2.

There has fortunately been a growing recognition of the importance of TAND, and interest in measuring TAND for clinical trial and clinical service delivery purposes. However, the multi-level nature has made it very difficult to measure and quantify TAND in a quick, affordable, yet robust and scientifically meaningful way. There are a number of key challenges contributing to the complexity of assessing and treating TAND. These will be discussed in more detail in chapter 3. In short, we propose that there has been a combination of poor awareness, a perceived complexity of TAND, and a consequential inability of clinical teams to identify and treat specific aspects of TAND. This has resulted in the under-identification and under-treatment of TAND around the globe.

The overarching thesis of the work presented here propose that TAND data may

show natural clustering and that by using data-driven strategies we may be able to identify a manageable number of natural TAND clusters. This in turn, we propose, would be able to improve assessment, intervention and education about TAND. Even though there have been no apparent clinical indications of natural clustering of TAND to date (Leclezio and de Vries, 2016), we based our hypothesis on observations of natural clustering in other model systems and genetic disorders. For instance, in a study of the behavioural phenotype of humans with Cornelia de Lange Syndrome (CdLS), categorical principal component analysis (PCA) was used as a data reduction tool and to describe relationships between a large number of behavioural variables (Wulffaert *et al.*, 2009). This methodology allowed for new insights into the relationships between physical and behavioural characteristics and into genotype-phenotype correlations.

In this study, we propose that multivariate data analysis techniques could be applied as a novel strategy in TSC to reduce the multi-dimensionality of data, and to identify natural TAND clusters. We posit that it would be of great significance to identify a small number of clusters that could help to reduce and make manageable the complexity of TAND. We also suggest that this strategy may, in future, be helpful both for the detection of aetiological subgroups or subtypes in TSC, and for a more personalised management and treatment approach in clinical settings.

1.4 Aims of the Thesis

The **general aim** of this study was to identify natural TAND clusters. The specific aims were:

- (a) To review the current knowledge about TAND and reflect on the factors that may contribute to the 'assessment and treatment gap' for TAND.
- (b) To pilot a range of statistical methodologies using multivariate data analysis techniques towards identification of natural TAND clusters.
- (c) To apply the pilot study methodology to a large sample to verify and expand pilot study findings towards identification of clinically-meaningful natural TAND clusters.

1.5 Author contributions to included manuscripts

Contributions to the three manuscripts included in this thesis have been authorised by the supervisor, Professor Petrus J de Vries (PdV). These manuscripts have also been approved by the University of Cape Town (UCT) doctoral degrees board as being appropriate for inclusion, as per UCT policy.

1. Leclezio, L. and de Vries, P.J. (2015). **Advances in the treatment of tuberous sclerosis complex** *Current Opinion in Psychiatry*. **28**, pp.113-120

I developed the search methodology applied in this review paper with the input from my supervisor, and subsequently conducted the database and journal searches. I mined the data, analysed the data and finally summarised all the data. I wrote the full first draft of the manuscript. My co-author, Professor de Vries, evaluated the draft and made theoretical and intellectual contributions. I made all revisions myself.

2. Leclezio, L. and de Vries, P.J. (2016). **Towards an improved understanding of TSC- associated neuropsychiatric disorders (TAND)**. *Advances in Autism*. **2**(2), pp.76-83

In this manuscript I combined a brief review of the multiple levels of TAND with a conceptual analysis of barriers and potential facilitators to assessment and intervention for TAND. My co-author, Professor de Vries reviewed the draft and made intellectual contributions. I made all revisions prior to publication myself.

3. Leclezio, L., Gardner-Lubbe, S., and de Vries, P.J. (2017). **Identification of natural clusters of TSC-Associated Neuropsychiatric Disorders - A Pilot feasibility study**. *Pediatric Neurology: Under Review*

I designed the pilot identification of natural TAND clusters under the influence of my supervisor, PdV. I collected the data and performed analysis with Sugnet Gardner-Lubbe (SGL). SGL wrote the code used in R. SGL and I ran the model and analysed the output data. All authors discussed the results and implications. I drafted the manuscript and made all revisions following review by SGL and PdV.

Chapter 2

ADVANCES IN THE TREATMENT OF TUBEROUS SCLEROSIS COMPLEX

Leclezio, L. and de Vries, P.J. (2015) *Current Opinion in Psychiatry*, **28**, 113-120

2.1 Introduction

Tuberous sclerosis complex (TSC) is a multi-system disorder that can affect virtually any organ system, with some manifestations more prevalent than others across the lifespan (Crino *et al.*, 2006; Curatolo *et al.*, 2008). The most common manifestations include benign tumours in the heart, kidneys, lungs, skin and brain. Often overlooked, TSC also includes a vast range of neuropsychiatric disorders, typically neurodevelopmental, behavioural and psychiatric difficulties. Significant phenotypic variability in the number and severity of physical features and neuropsychiatric manifestations are seen in TSC (Povey *et al.*, 1994; de Vries *et al.*, 2010; de Vries *et al.*, 2015a).

The disorder is caused by mutations in either of two genes, the *TSC1* gene on chromosome 9q34 (Povey *et al.*, 1994; van Slegtenhorst *et al.*, 1997) or the *TSC2* gene on chromosome 16p13.3 (The European Chromosome 16 Consortium, 1993; Povey *et al.*, 1994). The birth incidence of TSC is estimated to be 1 in 6,000 (Osborne *et al.*, 1991; O'Callaghan *et al.*, 1998; Thiele and Jozwiak, 2010) and seems to have an equal male/female distribution. Up to 75% of cases occur as spontaneous mutations and the remaining ~25% are inherited in an autosomal dominant manner (Crino *et al.*, 2006). Currently there is no known cure for Tuberous Sclerosis Complex. However, an understanding of the functional relationship between the TSC1-TSC2 complex and mTOR has led to important clinical advances in the use of mTOR inhibitors as molecularly targeted treatments of the mTOR overactivation caused by mutations in either the *TSC1* or

TSC2 gene (Povey *et al.*, 1994; van Slegtenhorst *et al.*, 1997). Given the multi-system nature of the disorder, appropriate management and coordination of specialist care is crucial across the lifespan to limit morbidity and mortality in this disease and to optimize quality of life (Krueger *et al.*, 2013).

Here we review three main themes that emerged from the TSC literature over the last 18 months. Firstly, the revised diagnostic criteria and surveillance/management guidelines (Krueger *et al.*, 2013; Northrup *et al.*, 2013). The revised criteria and monitoring guidelines emerged from the 2012 International TSC Consensus Conference. At the consensus conference, the Neuropsychiatry Panel coined a new term – Tuberous Sclerosis Associated Neuropsychiatric Disorders (TAND). We next review the two recent papers around TAND and a TAND Checklist developed by the Neuropsychiatry panel as a potential screening tool for TAND (de Vries *et al.*, 2015a; Leclezio *et al.*, 2015). Finally, in view of the rapid progress around molecularly targeted treatments of the physical manifestations of TSC, we discuss the advances and debates around molecular treatments for the neuropsychiatric features of the disorder (Bissler *et al.*, 2013; Franz *et al.*, 2013).

2.2 International TSC Consensus guidelines and recommendations

Lagos and Gomez, based at the Mayo Clinic reported in 1967 the findings from a five generation family of 71 individuals affected by TSC. These data led to the first set of diagnostic criteria proposed by Gomez. The so-called ‘Gomez’ criteria were revised in 1979 (Gomez, 1979). In 1998, the National Institutes of Health (NIH) sponsored a conference with the aim to develop consensus recommendations for diagnosis (Roach *et al.*, 1998) and clinical management of individuals with TSC (Roach *et al.*, 1998; Gomez *et al.*, 1999).

The last 15 years have however seen a number of significant advances in understanding the pathogenesis and potential treatments of TSC. The *TSC1* and *TSC2* genes had been identified just before the 1998 consensus conference, but the roles of the putative ‘tumour suppressor genes’ were unknown. In 2002/2003 the discovery was made that the *TSC1* and *TSC2* proteins interact with one another, and that they act as a key signalling protein in the PI3K-mTOR

intracellular signalling pathway. This immediately led to insight into the pathophysiological mechanism of TSC and showed that loss of either gene leads to overactivation of mTOR (Kennerson *et al.*, 2002; Tee *et al.*, 2002). At the time, rapamycin, the prototypical mTOR inhibitor, was already licensed by the FDA for use in organ transplant. Very rapidly, the TSC community therefore moved to clinical trials of mTOR inhibitors. The first phase III double-blind, placebo-controlled trials of everolimus, an mTOR inhibitor, were published and showed evidence of shrinkage of subependymal giant cell astrocytomas (SEGA) and of renal angiomyolipomas (Bissler *et al.*, 2013; Franz *et al.*, 2013). Everolimus was licensed for use in TSC in 2012 by the FDA and European Medicines Agency (EMA) (Talan, 2010).

Given the key advances in TSC, there was growing interest in convening a consensus conference to reassess the diagnostic criteria, to consider the role of mutational analysis in diagnosis, and in particular, to improve the monitoring and treatment guidelines for the disorder given evidence from clinical trials. The second International TSC consensus conference was held in June 2012 in Washington DC, and brought together 79 individuals from 14 countries to review and reach consensus on diagnostic, surveillance, and management recommendations for individuals with TSC (Northrup *et al.*, 2013; Krueger *et al.*, 2013). **Table 2.1** shows the updated diagnostic criteria for Tuberous Sclerosis Complex.

Table 2.1 Revised diagnostic criteria for tuberous sclerosis complex 2012, (Northrup *et al.*, 2013)

A. Genetic diagnostic criteria

The identification of either a *TSC1* or *TSC2* pathogenic mutation in DNA from normal tissue is sufficient to make a definite diagnosis of Tuberous Sclerosis Complex (TSC). A pathogenic mutation is defined as a mutation that clearly inactivates the function of the *TSC1* or *TSC2* proteins (e.g., out-of-frame indel or nonsense mutation), prevents protein synthesis (e.g., large genomic deletion), or is a missense mutation whose effect on protein function has been established by functional assessment. Other *TSC1* or *TSC2* variants whose effect on function is less certain do not meet these criteria, and are not sufficient to make a definite

diagnosis of TSC. Note that 10% to 25% of TSC patients have no mutation identified by conventional genetic testing, and a normal result does not exclude TSC, or have any effect on the use of clinical diagnostic criteria to diagnose TSC.

B. Clinical diagnostic criteria

Major features

1. Hypomelanotic macules (≥ 3 , at least 5-mm diameter)
2. Angiofibromas (≥ 3) or fibrous cephalic plaque
3. Ungual fibromas (≥ 2)
4. Shagreen patch
5. Multiple retinal hamartomas
6. Cortical dysplasias*
7. Subependymal nodules
8. Subependymal giant cell astrocytoma
9. Cardiac rhabdomyomatosis
10. Lymphangiomyomatosis (LAM)**
11. Angiomyolipomas (≥ 2)**

Minor Features

1. “Confetti” skin lesions
2. Dental enamel pits (>3)
3. Intraoral fibromas (≥ 2)
4. Retinal achromic patch
5. Multiple renal cysts
6. Nonrenal hamartomas

Definite diagnosis: Two major features or one major feature with ≥ 2 minor features

Possible diagnosis: Either one major feature or ≥ 2 minor features

* Includes tubers and cerebral white matter radial migration lines

** A combination of the two major clinical features (LAM and angiomyolipomas) without other features does not meet criteria for definite diagnosis

Some of the important changes in comparison to the 1998 diagnostic criteria included the addition of a genetic criterion for TSC. Tuberous sclerosis can therefore now be diagnosed in the presence of a definite pathogenic mutation (see **Table 2.1**). The clinical criteria have remained very similar, apart from subtle improvements in, for instance, the number and size of physical characteristics required to be regarded as a 'major' criterion. Another change was removal of the category of 'probable TSC'. Under the new diagnostic criteria, a diagnosis is either 'definite' when 2 or more major criteria, or 1 major and 2 or more minor criteria are present, OR is 'possible' when only 1 major or 2+ minor criteria are present.

The surveillance and management guidelines used an evidence-based approach to collect levels of evidence for monitoring and treatment, and has resulted in an extremely useful set of guidelines for use in clinical practice. The guidelines presented clear rationales for monitoring and presented well-balanced and well-considered recommendations for investigations and interventions (Krueger *et al.*, 2013).

2.3 TSC-Associated Neuropsychiatric Disorders (TAND)

A wide range of behavioural, psychiatric, intellectual, academic, neuropsychological and psycho-social concerns are observed in individuals with TSC. Some of the high frequency difficulties across these levels of investigation are shown in **Table 2.2**. The table also indicates the typical tools used to assess these levels and provides a summary of rates across difficulties.

Table2.2. TSC-Associated Neuropsychiatric Disorders

| Level of Investigation | Assessment tools | Examples | Rates | References |
|------------------------|---|--|--|--|
| 1. Behavioural | Direct observation, parent and carer surveys, rating scale measures | <ul style="list-style-type: none"> • Anxiety • Depressed mood • Overactivity, restlessness and impulsivity • Aggression • Temper tantrums • Self-injury • Social-communication difficulties (including poor eye contact, repetitive and ritualistic behaviours, speech & language delay) • Sleep <p><i>The significant variability in rates of behavioural difficulties can at least in part be explained by the differential rates between individuals with and without intellectual disabilities</i></p> | 41-56% 19-43% 36-73% 51-66% 47-70% 17-69% 20-86% 20-74% | 1,2 1,2 3 3,4 3,4 5 3,4 5 |
| 2. Psychiatric | DSM-IV/5 (APA, 1994) and/or ICD-10 (WHO, 1993) | <ul style="list-style-type: none"> • Autism Spectrum Disorders <p><i>Estimates of prevalence rates of Autism Spectrum Disorders (ASD) in individuals with TSC have been reported at much higher rates than expected, ranging from 17-61%. The variability in these rates can be explained by the definition of ASD being used (classic autism versus autism spectrum disorders), the methodology employed (clinic based studies versus population based) and the level of intellectual ability of participants. The most comprehensive and detailed population-based study using the ADOS and ADI-R showed that 26% of children with TSC across all levels of intellectual ability met criteria for classic infantile autism and that about 50% met criteria for an ASD.</i></p> <ul style="list-style-type: none"> • Attention Deficit Hyperactivity Disorder | 26-50% 30-55% | 5,6 3, 7-10 |

| | | | | |
|--------------------------|---|---|------------------------------------|-------------------------|
| | | <p><i>General population rates of ADHD vary significantly and much of this variability could be explained by the different methodologies employed by researchers. A systematic review and meta-analyses of 102 studies comprising 171,756 subjects from around the globe showed that the ADHD worldwide-pooled prevalence was 5.29%. In contrast to the general population rate, the prevalence of ADHD in TSC is as high as 50% in all individuals with TSC, and ~30% in those with normal IQ.</i></p> <ul style="list-style-type: none"> • Mood Disorders • Anxiety Disorders | <p>26-35%</p> <p>28-59%</p> | <p>9,11</p> <p>9,11</p> |
| 3. Intellectual | Standardised measures of general intelligence and adaptive behaviours | <ul style="list-style-type: none"> • Normal intellectual ability • Mild-moderate intellectual disability • Severe-Profound intellectual disability <p><i>The majority of individuals with TSC (~70%) will have intellectual ability that falls on a normal distribution similar to that of the general population except that the mean IQ score is shifted downward to 93 versus 100 in the general population.</i></p> | <p>50%</p> <p>~20%</p> <p>~30%</p> | 3,12 |
| 4. Academic / Scholastic | Standardised measures of reading, writing, spelling and mathematics | <ul style="list-style-type: none"> • Reading • Writing • Spelling • Mathematics <p><i>To date there have been no direct studies of the rates and types of learning disorders individuals with TSC suffer from. Rating scale measures suggest that at least 30% of children with TSC who have normal intellectual ability are at high risk of specific academic difficulties or learning disorders.</i></p> | | 3 |
| 5. Neuro - psychological | Formal neuropsychological tools | <ul style="list-style-type: none"> • Attentional deficits (sustained attention, dual tasking) • Memory deficits • Complex visuospatial deficits | 40-90% | 13,14 |

| | | | | |
|------------------|--|--|-----|------|
| | | <ul style="list-style-type: none"> • Executive deficits (planning, set shifting, sequencing, verbal fluency) • Specific Language deficits <p><i>The pattern and rates of neuropsychological skills in TSC varies significantly across age, and may vary based on measurement tools used. The rates reported above are all based on performance of children and adults with normal intellectual ability.</i></p> | 24% | 3,15 |
| 6. Psycho-social | Direct observation, parent & carer surveys, self report | <ul style="list-style-type: none"> • Self-esteem • Family stress • Sibling difficulties • Peer relationship difficulties <p><i>There have been no studies to date to quantify the rates of psycho-social difficulties in TSC.</i></p> | | |
| 7. Biological | Impact of the physical manifestations of TSC and other medical conditions on neuropsychiatric presentation | <ul style="list-style-type: none"> • Epilepsy • Antiepilepsy medication • Subependymal giant cell astrocytomas • Renal failure <p><i>Many biological factors may play a potential role in the bio-psycho-social presentation of an individual with TSC. The items listed above all have a strong correlation with TAND, and should be considered, particularly where there is a sudden change in any TAND characteristics.</i></p> | | |

References:

1. Lewis *et al.*, 2004; 2. Pulsifer *et al.*, 2007; 3. de Vries, 2010a; 4. Eden *et al.*, 2014; 5. de Vries *et al.*, 2007; 6. Bolton *et al.*, 2002; 7. Gillbert *et al.*, 1994; 8. Prather and de Vries, 2004; 9. Muzykewics *et al.*, 2007; 10. Polanczyk *et al.*, 2007; 11. Smalley *et al.*, 1992; 12. Joinson *et al.*, 2003; 13. de Vries *et al.*, 2009; 14. Tierney *et al.*, 2011; 15. Ridler *et al.*, 2007

Neuropsychiatric disorders seen in TSC are some of the top concerns for families and individuals with TSC, and have an enormous impact on quality of life. Taking together all the levels of investigation listed, around 90% of individuals with TSC will have one or more of these concerns during their lifetime (de Vries, 2010a; de Vries *et al.*, 2015a). A survey of Tuberous Sclerosis Association members in the United Kingdom in 2010 indicated that only 18% of individuals with TSC had ever received an assessment or treatment for neuropsychiatric disorders (de Vries *et al.*, 2015a). These results indicated a significant treatment gap of around 70%.

At the TSC consensus conference in 2012, the neuropsychiatry panel expressed concern about the enormous treatment gap, and about the lack of clarity and consistency in the use of terminology across the levels of neuropsychiatric investigation. For instance, it was clear from the literature that terms such as 'behavioural difficulties', 'neurocognitive issues', 'neurobehavioural problems', 'psychiatric disorders' and 'developmental disability' were all used interchangeably, even in relation to pre-clinical animal studies (de Vries *et al.*, 2015a).

In an attempt to increase awareness of the burden of these difficulties, to increase the likelihood of screening for these problems, and to standardise terminology, the Neuropsychiatry Panel decided to coin the term TAND, Tuberous Sclerosis-Associated Neuropsychiatric Disorders, and recommended that all individuals with TSC should be screened for TAND at least annually (de Vries *et al.*, 2015a; Krueger *et al.*, 2013). To facilitate this process of screening, the Neuropsychiatry Panel agreed to develop a TAND Checklist for use as a potential screening tool. The panel were clear that they did not want to generate a 'diagnostic' tool, but rather a screening 'Checklist' to guide healthcare teams in a systematic enquiry across the levels of neuropsychiatric investigation required in TSC. Checklists are typically aimed at reducing errors of omission and are generally easy to administer and understand (Scriven, 2005). From a practicability point of view, the panel aimed to develop a checklist that would be straightforward to administer, comprehensive and easy to understand by families and individuals with TSC.

2.4 The TAND Checklist

The TAND Checklist consist of a pen-and-paper, double-sided, 2 page form and contains a set of questions that can be used to guide a 10 minute conversation between an appropriate healthcare provider (e.g. clinic nurse, counsellor, doctor) and a parent, caregiver or individual with TSC.

The TAND Checklist includes 7 basic developmental milestones items, 3 items on current level of functioning, 38 items across behaviour (19 items), psychiatric diagnosis (6 items), perceived intellectual disability (3 items), scholastic difficulties (4 items), neuropsychological skills (6 items), and psycho-social functioning (3 items). The TAND Checklist also includes a parent/caregiver/self-rating of the impact of TAND (on a scale of 1-10), and a similar item where the healthcare provider who completes the TAND Checklist with the person provides an overall TAND impact score (on a scale of 1-10). Two items allow for prioritisation of next steps and addition of extra concerns. An individual's TAND profile may change over time, thus supporting the need for re-evaluation on a regular basis. The TAND Checklist is presented in the appendix of the thesis.

As a screening tool, it is important that the TAND Checklist is regarded to have *face validity* (seen by professionals and families as capturing the essential and important aspects of concern), *content validity* (judged by experts to cover the range and depth of neuropsychiatric concerns of relevance to TSC), and *transferability* (the ability of the tool to be used across different settings by different people). Pilot validation of the TAND Checklist was performed by Leclezio and colleagues in 2014. In the study mixed-methods were used across two stages. In stage 1 feedback was gathered on the Checklist from 20 international TSC experts and 42 parents/carers. The aim was to examine face and content validity. Stage 2 involved the administration of the refined TAND Checklist to 20 South African parents of individuals with TSC concurrently with four other validated assessment tools. The aim of this stage was to examine concurrent validity and to obtain qualitative feedback on face-to-face administration of the TAND Checklist. Expert clinicians as well as families rated the TAND Checklist to have good face and content validity as reflected in quantitative analysis which showed high overall mean and median scores of 4

and 5 (five being the maximum rating). Stage 2 results showed moderate to very good correlations across key domain and subdomain scores examined. The TAND Checklist behavioural domain scores correlated very strongly with the Strength and Difficulties Questionnaire scores, with a Spearman Rho of 0.83 ($p \leq 0.001$). On the neuropsychological subdomain of executive function, the TAND Checklist correlated strongly with that of the BRIEF behaviour rating index (Spearman Rho of 0.75 and $p=0.001$). These results suggest good concurrent validity with the other four assessment tools used. Findings suggested that this simple tool may be a helpful aide memoire in the identification and subsequent treatment of TAND. Results of other aspects of validity and qualitative feedback were used to shape the Checklist to be both clinically meaningful and a useful research tool for future studies (Leclezio *et al.*, 2015).

2.5 The hope of molecularly targeted treatments for the neuropsychiatric manifestations of TSC

Molecular treatments for the physical manifestations of TSC are now a reality, and the international consensus conference have recommended mTOR inhibitors as first-line treatment for renal angiomyolipoma larger than 3cm, and as a medical treatment option for subependymal giant cell astrocytomas not amenable to surgery (for instance, where anaesthetic is contra-indicated, where likelihood of resection is low, for bilateral SEGA etc) (Bissler *et al.*, 2013; Franz *et al.*, 2013; Krueger *et al.*, 2013). Both phase III trials reported in 2013 also observed systemic benefits, particularly in skin manifestations (Bissler *et al.*, 2013; Franz *et al.*, 2013).

Given the positive progress in mTOR inhibitor trials, there has been growing interest in the possibility of mTOR inhibitors to treat some of the neuropsychiatric manifestations of TSC. In 2007 de Vries and Howe suggested a direct molecular pathway from genes to cognition and neurodevelopment in TSC, and suggested that rapamycin and other mTOR inhibitors might therefore improve or reverse some of these deficits too (de Vries and Howe, 2007). There has been some support from the animal literature for this hypothesis, including studies that have shown reversal of learning and social deficits in TSC mouse models (Ehninger *et al.*, 2012; Sato *et al.*, 2012; Tsai *et al.*, 2012).

There are a number of phase II clinical trials underway examining the role of mTOR inhibitors for this purpose in humans (see clinicaltrials.gov). To date, there has only been one early-phase study of neuropsychological skills in humans (Davies *et al.*, 2011). In a phase II study using sirolimus, an mTOR inhibitor, to treat angiomyolipomas in individuals with TSC and/or sporadic lymphangiomyomatosis (LAM), neuropsychological tests of memory and executive skills were administered to the eight adult participants with TSC at baseline, 4 months and 12 months. Immediate recall memory ('free recall') improved in seven of the eight participants and deteriorated in the remaining patient. Immediate recognition memory remained unchanged in three of the TSC participants and deteriorated in the remaining five. Executive skills improved in five of the eight participants, remained unchanged in 2, and dropped in one participant. Examination of the graphic representation did not show any association between improvement or deterioration within participants. In terms of recall memory, it appeared that baseline performance might have predicted direction of change, but this did not appear to be the case for the executive skills (Davies *et al.*, 2011).

There is evidence from the animal literature that mTOR inhibition may not be inevitably positive. Rapamycin decreased neurodevelopmental outcomes in wild-type mice (Tsai *et al.*, 2013), and prenatal administration of rapamycin to a TSC mouse model was not associated with positive outcome on behaviour tasks, in contrast to post-natal administration of rapamycin (Way *et al.*, 2012).

In the field of TSC there is great interest in early and even pre-emptive treatment of individuals with TSC (Jozwiak *et al.*, 2011). Results from the animal models, and the very limited human data, however, suggest that great caution, and many further explorations are required to determine the optimal timing for mTOR inhibition in the growing brain, and to establish how to identify those who are likely to improve as opposed to deteriorate in neuropsychological (or other neuropsychiatric level) performance. We suggest that in the central nervous system, particularly the growing brain, a therapeutic model of 'optimal mTOR signalling' should be sought, both in terms of *optimal timing* of treatment, in relation to dynamic neurobiological processes, and in terms of *optimal level* of

inhibition, in relation to baseline mTOR activation and/or the dynamic demands of mTOR activation during neuropsychological task performance.

2.6 Key Points

- Revised diagnostic criteria for TSC, including a genetic criterion, and evidence-based guidelines for the surveillance and management of TSC were published in 2013.
- Even though 90% of individuals with TSC will have neuropsychiatric difficulties during their lifetime, only about 20% ever receive evaluation and treatment.
- The term TAND (TSC-Associated Neuropsychiatric Disorders) was coined as an umbrella term for the multiple and complex bio-psycho-social challenges seen in TSC.
- All individuals with TSC should be screened for TAND at least annually. A TAND Checklist was developed as a potential screening tool.
- In spite of the future promise of mTOR inhibitors to treat neuropsychiatric aspects of TSC, we propose a model of 'optimal mTOR signalling' in the therapeutic approach to the neuropsychiatric features of the disorder.

2.7 Conclusion

Here we reviewed three themes of recent development in TSC of relevance to psychiatry, neuropsychiatry and the mental health of individuals with TSC. The majority of individuals with TSC will have some neuropsychiatric problems in their lifetime, with lifetime prevalence rates in the region of 90%. The vast range of challenges seen in TSC that emerge and change over developmental time and across dimensions can make it very difficult to know how to prioritise clinical concerns in order to develop next step strategies. No obvious 'natural clusters' of TAND profiles have been identified to date, and this may be a fruitful future strategy to explore. It is possible that distinct clusters of TAND may lead to better aetiological subtyping, identify profiles of clinical needs, and may guide treatment decisions for individuals with the disorder.

2.8 Chapter summary

Tuberous Sclerosis Complex (TSC) is a multi-system genetic disorder with physical and neuropsychiatric manifestations and significant research progress has been made in recent years. Here we focus on key advances over the last 18 months. Three main themes were identified in the literature. Firstly, the diagnostic criteria and surveillance guidelines for TSC were revised, incorporating a genetic criterion alongside clinical criteria, and making a positive step towards evidence-based treatment of TSC. Secondly, a new term – TSC Associated Neuropsychiatric Disorders (TAND) – was introduced as an umbrella term for all possible neuropsychiatric difficulties seen in TSC, and a TAND Checklist was developed as a screening tool. Thirdly, the risks and benefits of molecularly targeted treatments of the neuropsychiatric manifestations of TSC are being debated. The updated diagnostic criteria and management guidelines, the new concept of TAND, and the TAND Checklist should lead to significant improvements in the quality of care for individuals with TSC. The promise of mTOR inhibitors and other molecular treatments are still to be confirmed. We suggest that great care should be taken to identify ‘optimal mTOR signalling’ in the therapeutic approach to the neuropsychiatric features of the disorder.

Chapter 3

TOWARDS AN IMPROVED UNDERSTANDING OF TSC-ASSOCIATED NEUROPSYCHIATRIC DISORDERS (TAND)

Leclezio, L. and de Vries, P.J. (2016) *Advances in Autism*, 2, 76-83

3.1 Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder with multi-system involvement (Crino *et al.*, 2006; Curatolo *et al.*, 2008; Curatolo *et al.*, 2015). The disorder can affect virtually any organ system in the body, with the most common physical manifestations being benign tumours in the central nervous system, skin, kidneys, heart, and lungs (Crino *et al.*, 2006; Curatolo *et al.*, 2008; Northrup *et al.*, 2013). There is significant phenotypic variability in the number and severity of physical features of the disorder (Povey *et al.*, 1994; Crino *et al.*, 2006; Curatolo *et al.*, 2008; Krueger *et al.*, 2013; Curatolo *et al.*, 2015). TSC has a clear age-related expression pattern, with some features more prevalent during infancy and childhood and others more likely to manifest during adolescence or in adulthood (Crino *et al.*, 2006; Curatolo *et al.*, 2008; Curatolo *et al.*, 2015). Appropriate management and coordination of medical specialist care is crucial across the lifespan to reduce morbidity and mortality in individuals with TSC (Krueger *et al.*, 2013, Curatolo *et al.*, 2015).

TSC is also associated with a vast range of neuropsychiatric disorders that include behavioural problems, neurodevelopmental disorders, and major psychiatric disorders. The term TSC-Associated Neuropsychiatric Disorders (TAND) was recently introduced as an umbrella term for all these potential difficulties (de Vries *et al.*, 2015a; Curatolo *et al.*, 2015). The majority of individuals with TSC will have some neuropsychiatric problems in their lifetime, with lifetime prevalence rates in the region of 90% (de Vries *et al.*, 2015a). TAND

also has an age-related expression pattern, thus supporting the need for regular screening and comprehensive evaluation of these difficulties as set out in the monitoring guidelines for TSC (de Vries, 2010a; Krueger *et al.*, 2013; Curatolo *et al.*, 2015; Kingswood and de Vries, 2015).

3.2 The Neuropsychiatric Phenotype of TSC

There is no doubt that the neuropsychiatric disorders seen in TSC are some of the top concerns for families and have an enormous impact on quality of life for all who live with the condition (de Vries *et al.*, 2015a; Curatolo *et al.*, 2015). Infants, children, adolescents and adults with TSC may present with a varied and variable range of neuropsychiatric challenges across multiple levels or dimensions. TSC is one of the medical conditions most strongly associated with autism and autism spectrum disorders. As a result, TSC has become a powerful model system to improve our understanding of autism in the context of TSC. For the purpose of this review, we did, however, not want to focus on autism spectrum disorders over and above the vast range of other neuropsychiatric difficulties that may be seen in addition to or instead of autism. **Table 3.1** shows some of the most common problems reported by families and observed by clinicians, indicating the multiple levels of difficulties, and providing a summary of rates across difficulties (Leclezio and de Vries, 2015). Even though the majority of individuals with TSC will have neuropsychiatric problems across the lifespan, the range and variability seen has led to a number of clinical and research challenges. Below, we outline some of the key challenges, before proposing steps towards an improved understanding of TSC-associated neuropsychiatric disorders (TAND).

Table 3.1. Multiple levels of TSC-Associated Neuropsychiatric Disorders (TAND) and reported rates (Adapted from Leclezio and de Vries, 2015).

| TAND Levels of investigation | Rates |
|---|---|
| 1. Behavioural | |
| <ul style="list-style-type: none"> • Anxiety • Depressed mood • Overactivity, restlessness and impulsivity • Aggression | <p>41-56%</p> <p>19-43%</p> <p>36-73%</p> <p>51-66%</p> |

| | |
|--|---|
| <ul style="list-style-type: none"> • Temper tantrums • Self-injury • Social-communication difficulties (including poor eye contact, repetitive and ritualistic behaviours, speech & language delay) • Sleep <p><i>The significant variability in rates of behavioural difficulties may be explained by the differential rates between individuals with and without intellectual disabilities</i></p> | <p>47-70%</p> <p>17-69%</p> <p>20-86%</p> <p>20-74%</p> |
| 2. Psychiatric | |
| <p>Autism Spectrum Disorders</p> <p><i>The variability in these rates can be explained by the definition of ASD being used (classic autism versus autism spectrum disorders), the methodology employed (clinic based studies versus population based) and the level of intellectual ability of participants.</i></p> <ul style="list-style-type: none"> • Attention Deficit Hyperactivity Disorder <p><i>The prevalence of ADHD in TSC is as high as 50% in all individuals with TSC, and ~30% in those with normal IQ.</i></p> <ul style="list-style-type: none"> • Mood Disorders • Anxiety Disorders | <p>26-50%</p> <p>30-55%</p> <p>26-35%</p> <p>28-59%</p> |
| 3. Intellectual | |
| <ul style="list-style-type: none"> • Normal intellectual ability • Mild-moderate intellectual disability • Severe-Profound intellectual disability <p><i>The majority of individuals with TSC (~70%) will have intellectual ability that falls on a normal distribution similar to that of the general population except that the mean IQ score is shifted downward to 93 versus 100 in the general population.</i></p> | <p>50%</p> <p>~20%</p> <p>~30%</p> |
| 4. Academic/Scholastic | |
| <ul style="list-style-type: none"> • Reading | |

3.3 The Assessment and Management Gap of TAND

One of the first challenges surrounding the neuropsychiatric difficulties of TSC is a global lack of assessment for these problems. In a survey of members of the United Kingdom Tuberous Sclerosis Association in 2010, only 18% of families surveyed had ever received any of the evaluations or treatments recommended in the 2005 TSC guidelines (de Vries *et al.*, 2005; de Vries *et al.*, 2015a). Similarly, in the international Tuberous Sclerosis registry to increase disease Awareness (TOSCA) natural history study that includes over 2,000 patients from 31 countries (Kingswood *et al.*, 2017), fewer than 40% of patients had ever had an intellectual ability assessment, and no data were available to determine whether the majority of patients had ever been assessed for autism spectrum disorder, ADHD, anxiety disorders or depression (de Vries *et al.*, 2015a). Given that more than 90% of all individuals with TSC are likely to have some of these challenges, the ‘assessment and treatment gap’ (the difference between clinical need and services provided) is therefore in excess of 70% (de Vries *et al.*, 2015a). This means that most people with TSC receive good physical evaluation and care, but only a small proportion ever receive good quality neuropsychiatric and neurodevelopmental assessment and support.

At the 2012 International TSC consensus conference, the neuropsychiatry panel expressed concern about the enormous treatment gap. The Panel commented that the ‘treatment gaps’ observed in TSC were similar to those observed in the Human Immunodeficiency Virus (HIV) community, where there used to be an overemphasis on physical treatment of HIV-positive individuals without consideration of the major neurocognitive and neuropsychiatric features of HIV (Antinori *et al.*, 2007). The HIV community introduced the concept of HAND (**HIV-Associated Neurocognitive Disorders**) as a strategy to raise awareness of such concerns. Inspired by the success of HAND, the term TAND (**TSC-Associated Neuropsychiatric Disorders**) was coined (de Vries *et al.*, 2015a). Given that an individual’s TAND profile may change over time, the neuropsychiatry panel recommended that all individuals with TSC should be screened for TAND at least annually (Krueger *et al.*, 2013; de Vries *et al.*, 2015a). In order to facilitate the screening process, a TAND Checklist was developed to guide healthcare teams in a systematic enquiry across the levels of neuropsychiatric investigation

required in TSC (de Vries *et al.*, 2015a; Leclezio *et al.*, 2015).

Leclezio *et al.* (2015) conducted pilot validation of the TAND Checklist and found that it showed good content validity, very good to excellent internal consistency, and strong correlations with external validation tools suggesting good external validity. Overall, the pilot validation suggested that the TAND Checklist could provide a useful research tool and screening measure in clinical settings.

The TAND Checklist is used in TSC clinics in various countries and has to date been translated into 2 other languages (Dutch and Swedish) and is in the process of being translated into German, French, Spanish, Portuguese, Hebrew, Mandarin and Afrikaans. A free, downloadable version of the TAND Checklist can be found at <http://dx.doi.org/10.1016/j.pediatrneurol.2014.10.004>.

3.4 Perceived Uniqueness of TAND Profiles

A second challenge for TAND is the perceived 'uniqueness' of individual TAND profiles. **Table 3.1** summarised the group-based findings, but at an individual level, almost any combination of TAND characteristics can be seen. To date there are no obvious clinical indications of natural clustering of the components of TAND (de Vries, 2010a), and each individual appears to present with their very own unique profile. On the one hand it seems appropriate to advocate a 'personalised medicine' approach, recognising each individual's unique profile or TAND 'signature'. On the other hand, this becomes a clinically very complex task that may be beyond the expertise of the majority of clinical teams. During the pilot validation of the TAND Checklist, Leclezio *et al.* (2015) gathered data from 20 South African participants on behavioural difficulties across the lifespan. **Figure 3.1** shows a graphic representation of the behavioural items from the TAND Checklist data of these 20 participants. Behavioural problems, such as aggression, anxiety or self-injury, are indicated as either present (black) or absent (white). At first glance (**Figure 3.1**, Panel A) no obvious pattern of behavioural challenges was observed across participants. On closer inspection (**Figure 3.1**, Panel B), when the same data were rearranged and subjects were grouped based on the number and clustering of behavioural problems, it looks as though some grouping or clustering may be present. For instance, there may be a highly

affected group (mostly black boxes) and a mostly mildly affected group (mostly white boxes) of individuals. In between, there may be a subset of individuals with prominent ADHD-related (attentional) difficulties, and a group with ASD-related (social-communication) problems but less prominent attention-related difficulties. Visual inspection also suggests that there may be a group of individuals with prominent mood swings, aggression and tantrums, independent of other behavioural symptoms. It is at present unknown whether data reduction methods could reduce TAND variables sufficiently to a manageable number of segmented profiles. A small enough number of TAND clusters (perhaps fewer than 10) that have reasonable face validity could streamline early identification of profiles, ascertain clinical needs, and inform management options.

Panel A

| | | TP001 | TP002 | TP003 | TP004 | TP005 | TP006 | TP007 | TP008 | TP009 | TP010 | TP011 | TP012 | TP013 | TP014 | TP015 | TP016 | TP017 | TP018 | TP019 | TP020 | |
|------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| Sleep | Sleep | | | | | | | | | | | | | | | | | | | | | |
| Food | Eating | | | | | | | | | | | | | | | | | | | | | |
| Mood | Anxiety | | | | | | | | | | | | | | | | | | | | | |
| | Depression | | | | | | | | | | | | | | | | | | | | | |
| | Shyness | | | | | | | | | | | | | | | | | | | | | |
| Aggression | Mood | | | | | | | | | | | | | | | | | | | | | |
| | Aggression | | | | | | | | | | | | | | | | | | | | | |
| | Temper | | | | | | | | | | | | | | | | | | | | | |
| ASD | Self-injury | | | | | | | | | | | | | | | | | | | | | |
| | Delayed Lang | | | | | | | | | | | | | | | | | | | | | |
| | Repet words | | | | | | | | | | | | | | | | | | | | | |
| | Eye contact | | | | | | | | | | | | | | | | | | | | | |
| | Peer Relations | | | | | | | | | | | | | | | | | | | | | |
| ADHD | Repeat Bhv | | | | | | | | | | | | | | | | | | | | | |
| | Rigid | | | | | | | | | | | | | | | | | | | | | |
| | Hyper | | | | | | | | | | | | | | | | | | | | | |
| | Attention | | | | | | | | | | | | | | | | | | | | | |
| | Restless | | | | | | | | | | | | | | | | | | | | | |
| | Impulsive | | | | | | | | | | | | | | | | | | | | | |

Panel B

| | | TP009 | TP002 | TP014 | TP011 | TP012 | TP007 | TP008 | TP016 | TP003 | TP010 | TP006 | TP015 | TP018 | TP013 | TP004 | TP019 | TP005 | TP001 | TP017 | TP020 | |
|------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| Sleep | Sleep | | | | | | | | | | | | | | | | | | | | | |
| Food | Eating | | | | | | | | | | | | | | | | | | | | | |
| Mood | Anxiety | | | | | | | | | | | | | | | | | | | | | |
| | Depression | | | | | | | | | | | | | | | | | | | | | |
| | Shyness | | | | | | | | | | | | | | | | | | | | | |
| Aggression | Mood | | | | | | | | | | | | | | | | | | | | | |
| | Aggression | | | | | | | | | | | | | | | | | | | | | |
| | Temper | | | | | | | | | | | | | | | | | | | | | |
| ASD | Self-injury | | | | | | | | | | | | | | | | | | | | | |
| | Delayed Lang | | | | | | | | | | | | | | | | | | | | | |
| | Repet words | | | | | | | | | | | | | | | | | | | | | |
| | Eye contact | | | | | | | | | | | | | | | | | | | | | |
| | Peer Relations | | | | | | | | | | | | | | | | | | | | | |
| ADHD | Repeat Bhv | | | | | | | | | | | | | | | | | | | | | |
| | Rigid | | | | | | | | | | | | | | | | | | | | | |
| | Hyper | | | | | | | | | | | | | | | | | | | | | |
| | Attention | | | | | | | | | | | | | | | | | | | | | |
| | Restless | | | | | | | | | | | | | | | | | | | | | |
| | Impulsive | | | | | | | | | | | | | | | | | | | | | |

Figure 3.1. The variability of TSC-Associated Neuropsychiatric Disorders (TAND). The figure shows the pattern of lifetime behavioural problems across a group of 20 individuals with TSC (used with permission from Leclezio *et al.*, 2015). Each column represents one individual and each row represents a specific behavioural problem. Black box = abnormal; white box = no abnormality reported. Data are first presented in the order of assessment in the study (Panel A) and appear random without any obvious patterns. In Panel B data were rearranged and subjects were grouped based on the number and clustering of behavioural problems, with results providing a clue that natural clusters of behaviours in TSC may be identifiable.

3.5 Treatment Paralysis in TAND

As raised earlier, the perceived uniqueness of each individual's TAND profile clearly points to the need for a personalised approach to evaluation and treatment. However, the vastness of possibilities may seem overwhelming to families, may make signposting to the most relevant services very challenging, and may require numerous highly-skilled professionals to assess and treat the individual, thus making the process either very complex or prohibitively expensive. Many clinicians who are experts in TSC do not feel they have the skills to assess and treat TAND. Conversely many neurodevelopmental and mental health experts do not feel that they have sufficient knowledge of TSC to lead assessment and treatment of those with the disorder. Together, this has very much led to what we refer to here as 'treatment paralysis'. Early results from the TOSCA natural history study suggest that treatment paralysis may be real in TSC. Baseline TAND data from the first 2093 patients in the TOSCA study showed an average age of diagnosis for autism spectrum disorders in TSC of 7 years 7 months (de Vries *et al.*, 2015a). It is likely that expert clinicians over-emphasised assessment and treatment of physical manifestations (perhaps particularly epilepsy) and that the very late diagnosis of ASD was attributable to diagnostic overshadowing or treatment paralysis. Another example comes from an earlier study of attention difficulties and ADHD in children with TSC where 11 out of 20 children in a research study met criteria for ADHD, but only 1 had ever been diagnosed and treated for ADHD (de Vries, Gardiner & Bolton, 2009). Needless to say, increased awareness of TAND should lead to improved assessment and treatment. The potential identification of natural clusters of TAND difficulties should further reduce the sense of diagnostic overwhelmedness and treatment paralysis of clinical teams.

3.6 Potential next steps to improve our understanding of TAND

There is no doubt that the neuropsychiatric disorders seen in TSC are some of the top concerns for families and have an enormous impact on quality of life for all who live with the condition. As outlined above, there is currently a large TAND 'treatment/identification gap', each individual appears to present with their own unique profile of TAND, and clinicians are paralyzed by the multi-dimensionality of

TAND and its management and treatment options. In response to the above, the 2012 TSC surveillance and management guidelines now recommend annual screening of TAND (Northrup *et al.*, 2013) and a TAND Checklist was developed (de Vries *et al.*, 2015a) and piloted (Leclezio *et al.*, 2015). The hope is that coining of the term TAND, development of a TAND Checklist and inclusion of TAND in the management guidelines will improve awareness of the need to assess everyone with TSC for neuropsychiatric difficulties.

As a next step, the evaluation and treatment of TAND may benefit from the search for 'natural clusters' – groups of neuropsychiatric manifestations across multiple levels - that could reduce the infinite and unique patterns observed into a small number of 'TAND clusters'. Machine-based pattern recognition techniques have been used across a range of animal models and humans to reduce the multi-dimensionality of behavioural characteristics to generate new clusters (Geng *et al.*, 2003; Wulffaert *et al.*, 2009; Budaev, 2010; Meager *et al.*, 2012). For example, Geng and colleagues studied the complex and multidimensional behavioural phenotypes of the nematode *C. elegans*. Multivariate data analysis was used to reduce the multi-dimensionality of data and to assess in a quantitative way the similarities between different behavioural phenotypes. Two hundred and fifty three (253) parameters were reduced into 39 features. These features classified into an optimal number of 6 clusters. Interestingly, the phenotypic clusters generated mapped well onto the underlying molecular defects of the different models (Geng *et al.*, 2003). In 2012 Meager and colleagues examined the behavioural phenotype of wild and hatchery-reared Atlantic cod and used principal component analysis (PCA) to reduce the multi-dimensionality of the data and factor analysis (FA) to generate clusters rather than one-dimensional behavioural phenotyping methods. They were able to identify 8 behavioural clusters with behavioural phenotypes distinct in wild and hatchery-reared fish. Here the interesting observation was that there was a distinct phenotype in the wild cod not seen in the hatchery-reared animals. In a study of the behavioural phenotype of humans with Cornelia de Lange Syndrome (CdLS), categorical PCA was used as a tool to reduce data and to describe relationships between a large number of behavioural variables (Wulffaert *et al.*, 2009). In their study, the methodology allowed them new insights into the relationships between physical and behavioural characteristics and into genotype-phenotype correlations. Taking

together the examples from nematode, fish, and humans with a specific genetic disorder, we suggest that reducing the multi-dimensionality of neuropsychiatric manifestations seen in TSC through various multivariate data analysis techniques may lead to identification of natural TAND clusters. Age and gender can be added into these analyses, thus allowing for the dynamic nature of the developmental needs of individuals with TAND. It would be of great significance to identify a small number of clusters that could help to reduce and make manageable the complexity of TAND.

After identification of TAND clusters, a prioritisation exercise with TSC community stakeholders to identify TAND clusters of greatest concern will be helpful. It will be important to check with individuals, families and carers whether these newly generated 'TAND clusters' make sense to them and have face validity. This will ensure that statistical research remains embedded in real world needs.

Priority TAND clusters can then be selected to generate a toolkit of treatment options. In various neurodevelopmental disorders such as ADHD and autism, and mental health disorders such as anxiety and depressive disorders, evidence-based guidelines for treatment exist. It is best practice to develop clinical guidelines and/or toolkits in partnership with families and individuals affected by a particular condition. In the UK, for instance, the National Autistic Society led in the development of the National Autism Plan for Children (NAPC) and the NICE guidelines for assessment and treatment of autism (NAS, 2003; NICE 2011). In recent years the concept of community-based participatory research has developed a formal framework for participation of 'users' of research findings to ensure that what is done in an academic/laboratory-based setting has a high likelihood of being translated into real benefits for real people in the real world (Wallerstein and Duran, 2006). Excellent evidence-based guidelines that currently exist, such as the NICE guidelines mentioned above, alongside other tried and tested resources could potentially be incorporated into a toolkit for TSC clinical teams. An ideal toolkit should contain evidence-based guidelines and practical strategies and tips for individuals with TSC across age and abilities.

3.7 Conclusion

There is growing recognition of the burden of TAND, and the importance to assess and treat individuals with TAND. We hope that the coining of the term TAND, recommending annual TAND screening and development of the TAND Checklist will help to reduce the assessment/treatment gap that currently exists. We propose that future research should focus on reducing the complexity of TAND by searching for natural TAND clusters and thereby reducing the overwhelming sense of 'uniqueness' of individual TAND profiles. If our hypothesis proves to be correct and natural TAND clusters do exist, this may inform further research into the mechanisms and molecular treatments of TAND across human and animal models. For instance, if TAND clusters were identified it would be very interesting to see if any correlated to particular genotypes, given the emerging genotype-phenotype correlations described by van Eeghen *et al.* (2012) and Wong *et al.* (2015). Finally, we propose that the development of a simple toolkit of evidence-based guidelines, information and tips to families and clinical teams, could go some way to reducing the treatment paralysis surrounding TAND.

3.8 Chapter Summary

Tuberous Sclerosis Complex (TSC) is associated with many learning, behavioural, neurodevelopmental and psychiatric difficulties. Over 90% of those with TSC will have some of these concerns, yet typically no more than 20% receive support and treatment. This paper provides an overview of TSC-Associated Neuropsychiatric Disorders (TAND), explores barriers to identification and management of TAND, and proposes possible next steps to improve assessment and treatment of TAND. The chapter combined a brief review of the multiple levels of TAND with a conceptual analysis of barriers and potential facilitators to assessment and intervention for TAND. Results suggest that the perceived uniqueness of TAND leads to treatment paralysis for most healthcare professionals, thus explaining the assessment and treatment gap seen for TAND. This may in part be due to the multi-dimensionality of TAND, and in part due to lack of access to clear, useful and evidence-based resources for TAND. Identification of natural TAND clusters through machine-based learning and data reduction methodologies may yield a manageable number of natural groups of TSC-related neuropsychiatric problems, for which a basic 'toolkit' of evidence-

based interventions could be developed. Families and clinicians will benefit from a toolkit of tried and tested resources and evidence-based information to guide further investigation and management of TAND. Even though individuals will have unique TAND profiles, there may be key natural TAND Clusters (combinations of behaviours across multi-dimensional levels) that will simplify and improve access to further evaluation, treatment and neuroscientific research.

IDENTIFICATION OF NATURAL CLUSTERS OF TSC- ASSOCIATED NEUROPSYCHIATRIC DISORDERS – A PILOT FEASIBILITY STUDY

Leclezio, L., Gardner-Lubbe, S., and de Vries, P.J. (2017).

Pediatric Neurology, under review

4.1 Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutations in either of two genes, the *TSC1* gene on chromosome 9q34 (Povey *et al.*, 1994; van Slegtenhorst *et al.*, 1997) or the *TSC2* gene on chromosome 16p13.3. (European Chromosome 16 Consortium, 1993; Povey *et al.*, 1994). Up to 75% of cases occur as spontaneous mutations and the remaining ~25% are familial (Kingswood and de Vries, 2015). The birth incidence of TSC is estimated to be 1 in 5,800 newborn babies (Osborne *et al.*, 1991; O’Callaghan *et al.*, 1998; Krueger *et al.*, 2013) with an equal male/female distribution. TSC is a multi-system disorder and can affect nearly any organ system, with most common physical manifestations being benign tumours in the central nervous system, skin, kidneys, heart, and lungs. There is significant phenotypic variability in the number and severity of physical features of the disorder (Povey *et al.*, 1994; Curatolo *et al.*, 2015). TSC has an age-related expression pattern with certain features more prevalent during infancy and childhood, and others more likely to manifest during adolescence or in adulthood (Curatolo *et al.*, 2008). Management and coordination of medical specialist care is crucial across the lifespan to limit morbidity and mortality in individuals with TSC (Krueger *et al.*, 2013; Curatolo *et al.*, 2015).

Tuberous Sclerosis is associated with a vast and variable range of TSC-

Associated Neuropsychiatric Disorders (TAND) that can present in infancy, childhood, adolescence or adulthood. The multi-level manifestations of TAND include behavioural, psychiatric, intellectual, academic, neuropsychological and psycho-social difficulties. **Table 4.1** contains the various aspects of TAND, indicating the specific variables and domains examined in this pilot study.

Table 4.1. Components of TSC-Associated Neuropsychiatric Disorders (* indicates items included in this pilot study)

| TSC-Associated Neuropsychiatric Disorders (TAND) | | | | | |
|--|--------------------------|------------------------------|--------------|-------------------------|---------------------------|
| Behaviour | Psychiatric | Intellectual | Academic | Neuro-psychological | Psycho-Social |
| *Aggression | Autism spectrum disorder | Intellectual disability | *Reading | *Sustained attention | Self-esteem |
| *Temper tantrums | ADHD | Uneven intellectual profiles | *Writing | *Dual-tasking | Self-efficacy |
| *Anxiety | Anxiety disorder | | *Mathematics | Attentional switching | Parental stress |
| *Depressed mood | Depressive disorder | | *Spelling | *Memory recall | Relationship difficulties |
| *Self-injury | | | | *Spatial working memory | |
| *Inattention | | | | Cognitive flexibility | |
| *Hyperactivity | | | | | |
| *Impulsivity | | | | | |
| *Language delay | | | | | |
| *Poor eye contact | | | | | |
| *Repetitive behaviours | | | | | |
| *Sleep problems | | | | | |

In a recent conceptual review of TAND, three clinical and research challenges were identified (Leclezio and de Vries, 2015). The first challenge is a so-called ‘assessment and management gap’ of TAND. Despite the fact that the lifetime prevalence of TAND is in the region of 90% (de Vries *et al.*, 2015a), there appears to be a global lack of assessment for these problems. A study conducted in the United Kingdom in 2010 showed that only 18% of all families had ever received any of the evaluations or treatments recommended in the 2005 TSC guidelines (de Vries *et al.*, 2005; de Vries *et al.*, 2015a). Interestingly, in the international Tuberous Sclerosis registry to increase disease Awareness (TOSCA) study of over 2000 patients from 31 countries, fewer than 40% of patients had ever had an intellectual ability assessment, and very high rates of missing TAND data were reported (Kingswood *et al.*, 2017). These findings suggested that, even in expert TSC centres, TAND are likely to be under-

identified and under-treated.

In order to raise awareness of these neuropsychiatric difficulties in TSC and encourage assessment and screening, the term 'TAND' was coined (de Vries *et al.*, 2015a) and the current TSC surveillance and management guidelines (Krueger *et al.*, 2013) recommend annual screening for TAND. To guide healthcare teams in a systematic enquiry of TAND, a TAND Checklist was developed and pilot validated (de Vries *et al.*, 2015a; Leclezio *et al.*, 2015). Results from this pilot study suggested the TAND Checklist to be a good screening tool to identify possible neuropsychiatric difficulties (Leclezio *et al.*, 2015). A free downloadable English version of the TAND Checklist is available (de Vries *et al.*, 2015a) and a global programme to translate the TAND Checklist is currently underway.

The second challenge for TAND described by Leclezio & de Vries is the perceived “uniqueness” of individual TAND profiles (Leclezio and de Vries, 2015). It appears that, at an individual level, each person has their own set and combination of TAND features with no two individuals presenting the same TAND “signature”. This uniqueness poses significant challenges for diagnostic work-up, psycho-education and intervention planning, particularly given the rarity of TSC and the multi-level nature of TAND. Leclezio and de Vries commented that, to date, there have been no obvious indications of natural clustering, that is, natural, predictable grouping of specific neuropsychiatric characteristics across levels of TAND (Leclezio and de Vries, 2015). The authors suggested that the potential identification of such natural TAND clusters may be a powerful strategy to manage the assessment/management gap, and to reduce the third challenge, referred to as ‘treatment paralysis’ (Leclezio and de Vries, 2015).

In this study, we examined the feasibility of using multivariate data analysis techniques as a novel strategy in TSC to reduce the multidimensionality of data, in search of possible natural TAND clusters. We proposed that data reduction may identify a small number of TAND clusters that could help to reduce and make manageable the complexity of TAND. This strategy may be helpful both for the detection of aetiological subgroups or subtypes in TSC, and for a more personalised management and treatment approach in clinical settings. Our aims

were to investigate the practicability of identifying natural TAND clusters and to identify suitable multivariate data analysis techniques for larger-scale study.

4.2 Methods

4.2.1 Subjects

Participants for this study were part of the 'Pilot Validation of the Tuberous Sclerosis-Associated Neuropsychiatric Disorders (TAND) Checklist' study (Leclezio *et al.*, 2015). Twenty study participants from Cape Town, South Africa, were recruited through the Red Cross War Memorial Children's Hospital TSC Clinic. Thirty-seven study participants from Australia were recruited via the Australasian Tuberous Sclerosis Society. To be eligible, participants had to meet criteria for TSC (Northrup *et al.*, 2013) and had to have a parent or caregiver who could complete the TAND Checklist in English. The pilot data deliberately included participants with a wide age and ability range.

4.2.2 Procedures

The TAND Checklist was administered to parents and caregivers of individuals with TSC by one of the researchers (LL or PJdV). The TAND Checklist follows the neuropsychiatric levels of investigation outlined previously (de Vries *et al.*, 2015a) and contains the following 12 sections: 1. Basic developmental milestones; 2. Current level of functioning; 3. Behavioural concerns; 4. Psychiatric disorders diagnosed; 5. Intellectual ability; 6. Academic skills; 7. Neuropsychological skills; 8. Psychosocial functioning; 9. Parent, caregiver, or self-rating of the impact of TAND; 10. Prioritisation list; 11. Additional concerns; and 12. Healthcare professional rating of the impact of TAND. Questions require simple YES or NO responses to most sections (de Vries *et al.*, 2015a).

4.2.3 Data Analysis

All analyses were performed with the R software package (R Core Team, 2016). The following sections of the TAND Checklist were included in the analysis: Section 3, behavioural challenges (19 questions/variables); Section 5, academic skills (4 variables); and Section 7, neuropsychological skills (6 variables). Given that all variables were binary (Yes/No), model-based clustering assuming an underlying Gaussian distribution was not considered suitable. Instead, the mean

squared contingency coefficient (Cramer, 1999) was used to compute a correlation matrix for the 29 variables selected from the TAND Checklist. Where missing values were present, these were omitted pairwise in correlation computations.

Several clustering solutions were compared. Hierarchical clustering methods provide a clustering tree visually representing the merging of TAND variables and suggesting a suitable number of clusters. Complete linkage, average linkage, WARD's method and McQuitty's methods were applied with the `hclust()` R function. Although hierarchical clustering has often been used with great success, the algorithm is fairly naïve and some more recent methods in the R package `cluster` (Maechler *et al.*, 2016) were therefore also investigated. PAM (partitioning around medoids) is an extension of the popular k-means clustering method. K-means could not be applied given that it required a numerical data matrix rather than a dissimilarity matrix based on the square contingency coefficient correlation matrix. The FANNY (fuzzy clustering) method allocates a probability for belonging to each cluster rather than simply allocating each item to a single cluster. DIANA (divisive analysis), also a hierarchical clustering method, was also used. In contrast to the other methods where larger clusters are formed by merging smaller clusters, this method forms smaller clusters by dividing larger clusters.

After a suitable clustering solution was obtained, exploratory factor analysis was employed with the function `fa()` from the R package `psych` (Revelle, 2016). The factor analysis was also performed on the means squared contingency coefficient correlation matrix. All the different options of factor extraction and rotation available in the `fa()` R function were investigated. These combinations were applied to solutions with between four and seven factors. In order to find the factor solution that best matched the cluster analysis solution, the Tucker index of factor congruence (Lorenzo-Seva and Ten Berge, 2006) was used. To summarise the congruence from the matrix of indices, the matrix was optimally rotated to the indicator matrix with Orthogonal Procrustes Analysis, and the differences in trace were computed. For algebraic details, see **Appendix D**.

Finally, we set out to compare the data-driven cluster solutions with the exploratory factor analysis in order to examine similarities and differences of the

two approaches.

4.3 Results

4.3.1 Cluster Analysis

Cluster analysis showed a six cluster solution to be optimal. Hierarchical clustering with WARD's method produced the most suitable six cluster solution. A dendrogram of the WARD cluster analysis shows detail of the natural clustering of the TAND variables examined (**Figure 4.1**). A heatmap of the WARD cluster analysis results (**Figure 4.2**) shows TAND variables of study participants across the six clusters.

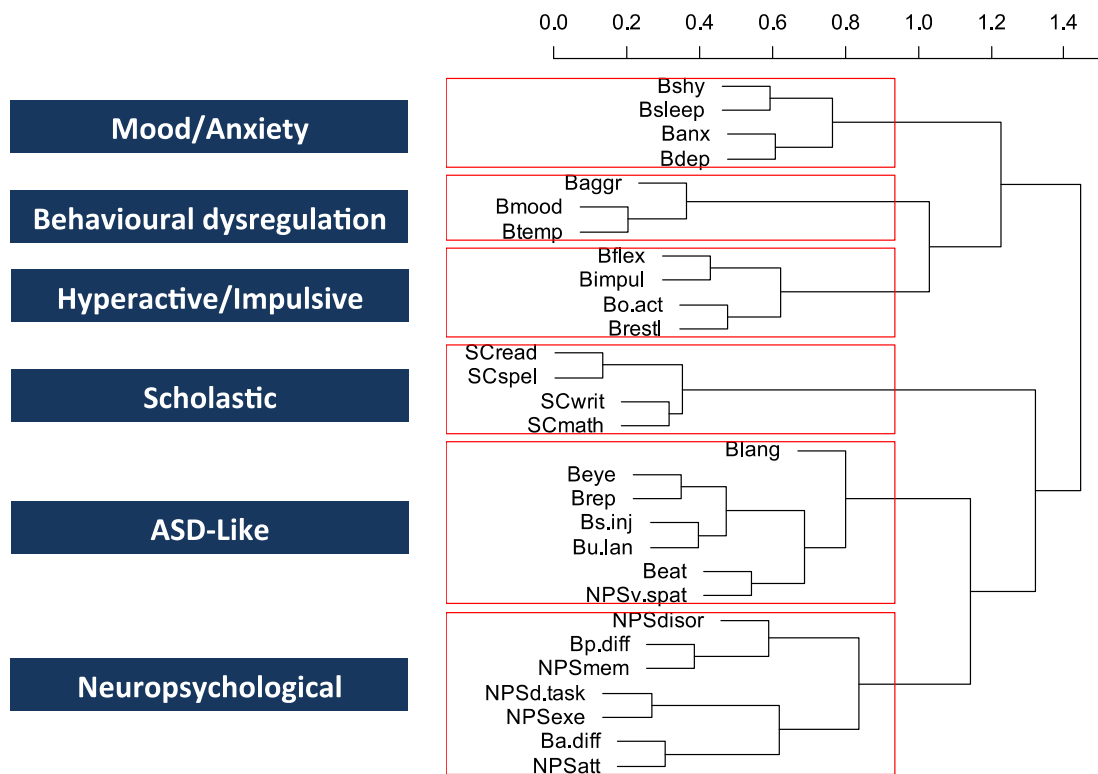


Figure 4.1. Hierarchical cluster analysis with WARD's methods produced six clusters.

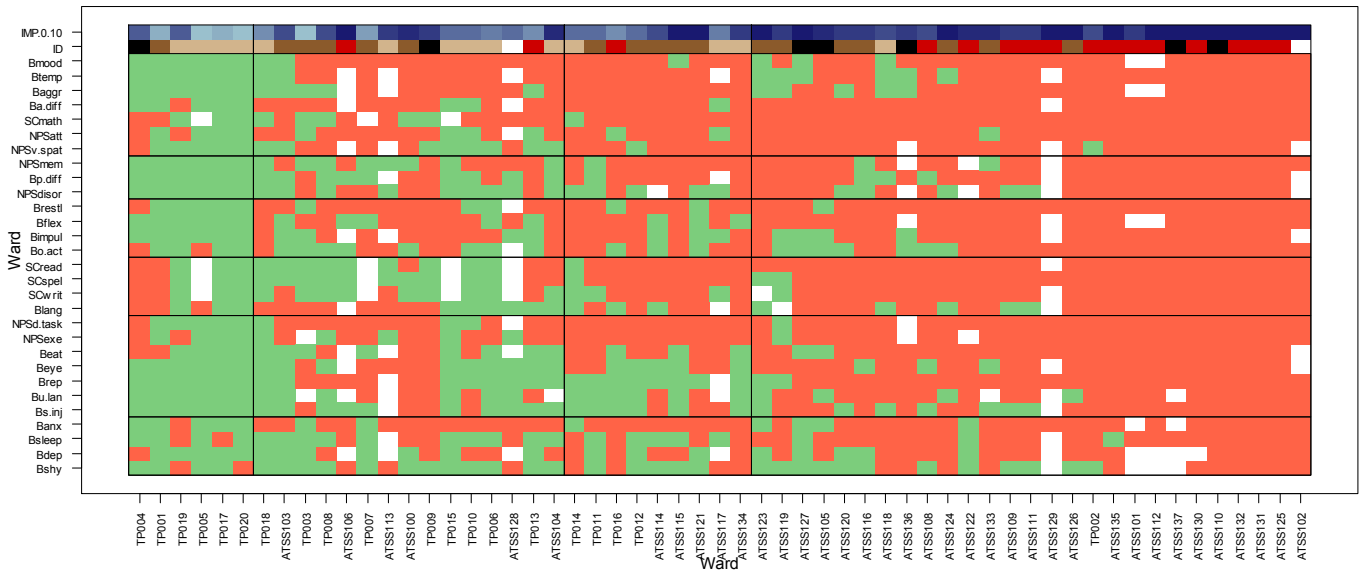


Figure 4.2. WARD's cluster analysis results show TAND variables across in the six clusters (Y-axis) for all study participants (X-axis) in a heatmap.

RED = Yes response to TAND Checklist item

GREEN = No response to TAND Checklist item

WHITE = Missing item

As shown in the dendrogram, the first cluster included difficulties with reading, spelling, writing, and mathematics, suggesting a natural 'Scholastic' cluster. The second cluster included delayed language, unusual eye contact, repetitive behaviours, unusual use of language, self-injurious behaviours, difficulties associated with eating (or unusual food preferences), and visio-spatial difficulties. These characteristics suggested a natural 'Autism Spectrum Disorder-like' cluster. The third cluster included aggressive outbursts, mood swings, and temper tantrums, suggesting a natural 'Dysregulated Behaviour' cluster. The fourth included memory problems, dual-task deficits, executive deficits, daily life attention difficulties, neuropsychological attention difficulties as well as disorientation and peer difficulties. These suggested a natural 'Neuropsychological' cluster. The fifth cluster included overactivity, restlessness, impulsivity and inflexibility, suggesting a 'Hyperactive/Impulsive' cluster. The sixth cluster included anxiety, depression, extreme shyness and sleep difficulties, suggesting a natural 'Mood/Anxiety' cluster.

The heatmap (**Figure 4.2**) shows these 6 clusters across individual participants in the study. Results showed a group of individuals who didn't fall into any clusters (not affected by set variables), some that fell into 1 or 2 clusters, and a group of individuals affected across most or all clusters.

4.3.2 Exploratory Factor Analysis

As shown in **Table 4.2** the factor analysis solution which most closely matched WARD's hierarchical cluster analysis was the least squares extraction method with Bentler's T orthogonal rotation (Benedik *et al.*, 1977) which also produced six factors. The six factors also mapped onto clinical constructs linked to scholastic skills, ASD, dysregulated behaviour, neuropsychological deficits, hyperactive/impulsive behaviours and a mixed/mood factor.

Table 4.2. Exploratory factor analysis produced six factors mapping onto clinical constructs identified.

| | | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|----------------------------------|-----------|----------|----------|----------|----------|----------|----------|
| Scholastic | SCspel | 0.901 | 0.102 | 0.172 | | 0.163 | |
| | SCread | 0.852 | 0.238 | | | 0.164 | |
| | SCmath | 0.851 | | 0.137 | | | 0.119 |
| | SCwrit | 0.848 | 0.123 | | 0.177 | | |
| | Bs.inj | 0.169 | 0.804 | 0.236 | | -0.123 | |
| ASD-Like | Beye | 0.106 | 0.795 | | 0.265 | 0.127 | |
| | Brep | 0.151 | 0.732 | 0.144 | 0.27 | 0.251 | |
| | Bu.lan | 0.284 | 0.634 | 0.245 | 0.119 | | |
| | NPSdisor | 0.26 | 0.521 | | 0.367 | -0.121 | |
| | Beat | 0.262 | 0.491 | 0.106 | | 0.42 | 0.281 |
| | NPSv.spat | 0.401 | 0.482 | | 0.298 | 0.192 | 0.148 |
| | Bp.diff | 0.338 | 0.471 | 0.31 | 0.373 | -0.219 | 0.155 |
| | Blang | 0.206 | 0.469 | | 0.177 | 0.24 | -0.467 |
| Behavioural dysregulation | Btemp | 0.155 | 0.14 | 0.888 | 0.179 | 0.126 | |
| | Bmood | | 0.318 | 0.791 | | | 0.168 |
| | Baggr | 0.337 | 0.117 | 0.738 | | 0.187 | |
| | Banx | 0.112 | -0.102 | 0.442 | 0.207 | 0.243 | 0.438 |
| | Ba.diff | | 0.224 | 0.235 | 0.844 | | |
| Neuropsychological | NPSatt | 0.12 | 0.164 | | 0.763 | 0.312 | |
| | NPSd.task | 0.436 | 0.203 | 0.343 | 0.628 | 0.123 | |
| | NPSexe | 0.461 | 0.144 | 0.386 | 0.522 | | |
| | Bo.act | 0.265 | | 0.117 | | 0.753 | |
| Hyperactive/Impulsive | Brestl | 0.271 | 0.15 | | 0.42 | 0.644 | |
| | Bimpul | | | 0.489 | 0.322 | 0.623 | 0.106 |
| | Bflex | | 0.427 | 0.341 | | 0.53 | 0.169 |
| | Bshy | | 0.155 | | | 0.111 | 0.699 |
| Mood/Mixed | Bdep | 0.224 | | 0.307 | 0.162 | 0.122 | 0.636 |
| | Bsleep | | 0.406 | | 0.275 | | 0.605 |
| | NPSmem | 0.456 | 0.178 | 0.246 | 0.348 | -0.231 | 0.472 |

4.3.3 Comparison of WARD's cluster analysis and Factor analysis

Figure 4.3 shows the similarities and differences between cluster analysis and exploratory factor analysis. In the "Scholastic" natural TAND cluster there was a

perfect match of identified variables (spelling, reading, mathematics, and writing) between cluster and factor analysis methods. In the second “ASD-like” natural TAND cluster, factor analysis included two additional characteristics, peer difficulty and disorientation, but other items were identical. In the “Dysregulated Behaviour” natural TAND cluster, factor analysis added anxiety behaviours to the other three variables (temper tantrums, mood swings and aggressive outbursts). Factor analysis identified an attentional-executive “Neuropsychological” natural TAND cluster, while cluster analysis also included memory difficulties, peer difficulties, and disorientation in this cluster. The “Hyperactive/impulsive” natural TAND cluster showed a perfect match between cluster and factor analysis methods (over-activity, restlessness, impulsivity, and inflexibility). In the “Mood/Mixed” natural TAND cluster, factor analysis combined depressed mood, sleep problems, extreme shyness and memory difficulties, whereas cluster analysis included anxiety in the “Mood” cluster, and clustered memory difficulties in the “Neuropsychological” cluster.

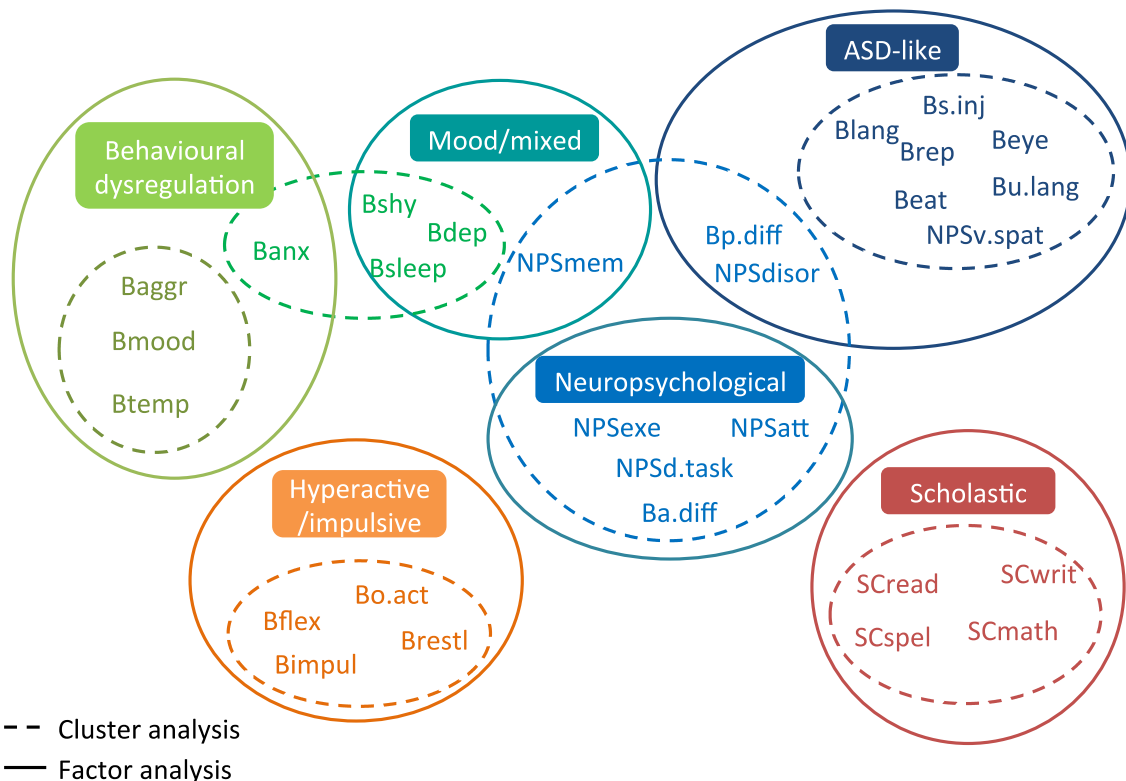


Figure 4.3. Comparison of WARD’s cluster analysis and exploratory factor analysis shows the similarities and differences in the six TAND clusters identified.

4.4 Discussion

The complexity and apparent individual ‘uniqueness’ of TSC-associated neuropsychiatric (TAND) profiles in people with TSC has meant that most people never received appropriate assessment or intervention for the range of neuropsychiatric manifestations that they may have had. We proposed that identification of relatively small number of natural TAND clusters might improve assessment and intervention for individuals with TAND. In this first ever study to investigate the occurrence of natural TAND clusters, we explored the practicability of applying data-driven strategies using the TAND Checklist (de Vries *et al.*, 2015a). Using data across 29 variables from 56 unrelated individuals with TSC, the multivariate cluster analysis and factor analysis methods applied generated 6 natural TAND clusters with clinically-meaningful face validity.

Based on the existing TSC literature, the Scholastic cluster maps very well onto the high rates of academic difficulties and learning disorders seen in TSC (Joinson *et al.*, 2003; de Vries, 2010a). The Hyperactive/Impulsive cluster represents another commonly reported set of difficulties, with very high rates of ADHD reported in TSC (Prather and de Vries, 2004; de Vries, 2010a). TSC is one of the medical conditions most commonly associated with ASD (Bolton *et al.*, 2002; de Vries, 2010), and the ASD-like cluster therefore seems intuitively meaningful. Many individuals with TSC, particularly those with normal intellectual ability have high rates of neuropsychological deficits in areas such as attentional-executive skills and memory (Prather and de Vries, 2004; Ridler *et al.*, 2007; de Vries *et al.*, 2009; de Vries, 2010a), so the natural clustering of neuropsychological deficits therefore also appears meaningful. It was interesting to observe the emergence of a cluster of behavioural difficulties including aggression, mood swings and temper tantrums. We suggest that this may point towards the existence of a ‘Dysregulated Behaviour’ cluster in TSC. The manifestations of anger, temper tantrums and mood swings are certainly clinically common and highly problematic to families, and correlate with other behaviours that challenge (Prather and de Vries, 2004; Eden *et al.*, 2014), suggesting this to be a potentially important natural TAND cluster to explore further. Whilst anxiety and depressed mood is very common in TSC (de Vries, 2010a), the Mood/Mixed cluster identified requires further exploration.

The second aim of this study was to identify suitable methods of data reduction. In this pilot study, WARD's hierarchical cluster analysis produced the most appropriate six cluster solution with good clinical face-validity. After identification of the six cluster solution, exploratory factor analysis was applied, and rendered very similar results to those generated through cluster analysis. The comparability between the two methods points towards robustness in the natural clustering of the TAND variables examined here. The minor differences between the cluster and factor analysis, however, suggest that it would be prudent to combine these methods in next-step examination of TAND.

4.4.1 Limitations

Firstly, we are conscious that there are many potential limitations to these pilot findings, and we remain cautious not to over-interpret results based on the small sample of participants presented here. However, we were encouraged to see the emergence of six potential natural TAND clusters from the data. Replication and extension in a larger sample should produce more definitive findings. Secondly, the TAND Checklist was used here to identify potential natural TAND clusters in this pilot study. No other sources of information that may be relevant in cluster analysis or factor analysis, such as clinical evaluations or neuropsychological assessments were included. We acknowledge that it is therefore theoretically possible that other clusters and factors may be identified using different kinds of multi-level data. However, we specifically wanted to use the TAND Checklist here, given that it is simple, yet systematic, and freely available. As reported earlier, pilot validation of the TAND Checklist (Leclezio *et al.*, 2015) indicated that the TAND Checklist was a valid tool in extrapolating multi-level neuropsychiatric manifestations in TSC. Thirdly, we acknowledge that some participants were recruited through a parent organisation, which might have introduced biases. However, the purpose here was an exploratory methodological evaluation, and the clinical significance of findings should therefore be deferred until replication and elaboration is performed in a larger sample.

4.5 Conclusion

The need for identifying natural TAND clusters to reduce the perceived overwhelming uniqueness of TAND profiles has gained urgency with the parallel development of tools such as the TAND Checklist for screening neuropsychiatric manifestations in TSC. This pilot feasibility study was the first of its kind to apply multivariate data analysis techniques to identify natural TAND clusters. WARD hierarchical cluster analysis rendered six clinically-meaningful clusters and factor analysis produced similar results. Larger-scale replication and extension is now required. Factor analysis can potentially also be used to generate ‘factor scores’ for each individual on a specific factor, thus leading to a ‘personalised’ quantification of an individual’s natural TAND cluster profile. We hope that successful next steps may also facilitate the development of resources, guidelines and training to improve the assessment and treatment of TAND in individuals with TSC.

4.6 Chapter Summary

Tuberous Sclerosis Complex (TSC) is a genetic disorder with multi-system involvement. The lifetime prevalence of TSC-Associated Neuropsychiatric Disorders (TAND) is in the region of 90% in an apparently unique, individual pattern. This ‘uniqueness’ poses significant challenges for diagnosis, psycho-education, and intervention planning. To date, no studies have explored whether there may be natural clusters of TAND. The purpose of this pilot study was (a) to investigate the practicability of identifying natural TAND clusters, and (b) to identify appropriate multivariate data analysis techniques for larger-scale studies. TAND Checklist data were collected from 56 individuals with a clinical diagnosis of TSC (n= 20 from South Africa; n = 36 from Australia). Using R, the open-source statistical platform, mean squared contingency coefficients were calculated to produce a correlation matrix, and various cluster analyses and exploratory factory analysis (EFA) were examined. WARD’s method rendered six TAND clusters with good face validity and significant convergence with a six-factor EFA solution. The data-driven strategies identified a ‘Scholastic’ cluster of TAND manifestations, an ‘ASD-like’ cluster, a ‘Dysregulated Behaviour’ cluster, a ‘Neuropsychological’ cluster, a ‘Hyperactive/Impulsive’ cluster, and a

'Mixed/Mood' cluster. These pilot results suggest that a combination of cluster analysis and exploratory factor analysis methods may be able to identify clinically-meaningful natural TAND clusters. Findings require replication and expansion in larger dataset, and could include quantification of cluster/factor scores at an individual level.

MULTIVARIATE DATA ANALYSIS IDENTIFIES NATURAL CLUSTERS OF TUBEROUS SCLEROSIS COMPLEX ASSOCIATED NEUROPSYCHIATRIC DISORDERS (TAND)

5.1 Introduction

As illustrated in the previous chapters, each person with TSC appears to present with their own unique profile or 'TAND signature' with no two individuals presenting the same combination of TAND features (Leclezio and de Vries, 2016). We suggested that the complexity and perceived uniqueness of TAND profiles as a barrier to diagnostic work-up, psycho-education and intervention planning, and proposed that the identification of natural TAND clusters - predictable groupings of specific neuropsychiatric characteristics - may be a powerful strategy to reduce the perceived overwhelming uniqueness of individual TAND profiles and resulting treatment paralysis, and could lead to the development of a personalised approach to management and treatment of individuals with TSC in clinical settings.

Machine-based data reduction methods have been used in humans to reduce the multi-dimensionality of behavioural characteristics to identify previously unrecognised clusters of behaviours. For example, in a study of the behavioural phenotype of humans with Cornelia de Lange Syndrome (CdLS), categorical principal component analysis (PCA) was used as a data reduction tool and to describe relationships between a large number of behavioural variables (Wulffaert *et al.*, 2009). This methodology allowed for new insights into the relationships between physical and behavioural characteristics and into genotype-phenotype correlations.

In a pilot feasibility study, Leclezio *et al.* (2017) set out to examine the viability of using multivariate data analysis techniques as a novel strategy in TSC to search

for possible natural TAND clusters. Cluster analysis of 29 variables in 56 individuals rendered six natural TAND clusters with good face validity and significant convergence with a six-factor exploratory factor analysis solution (Leclezio *et al.*, 2017). The natural TAND clusters identified included a 'Scholastic' cluster of TAND manifestations, an 'ASD-like' cluster, a 'Dysregulated Behaviour' cluster, a 'Neuropsychological' cluster, a 'Hyperactive/Impulsive' cluster, and a 'Mixed/Mood' cluster. The pilot methodological study, however, had a small sample from only two centres (South Africa and Australia) and, whilst informative, therefore clearly required replication and expansion. Additional unanswered questions from this study included statistical robustness and internal consistency of the putative TAND clusters.

In this study, our aim was to determine whether multi-dimensional TAND data could be reduced to clinically meaningful, natural TAND clusters in a much larger dataset. In addition, we set out to examine robustness and internal consistency of identified natural TAND clusters.

5.2 Methods

5.2.1 Subjects

Participants for this study (n=453) were recruited from five global TSC clinics: Cincinnati, USA (365 participants), Boston, USA (25 participants), Brussels, Belgium (25 participants), Dallas, USA (14 participants) and Leuven, Belgium (9 participants). An additional n=16 participants were recruited through Tuberous Sclerosis International (TSCi). To be eligible, participants had to meet criteria for TSC (Roach *et al.*, 1998, Krueger *et al.*, 2013). The data deliberately included participants with a wide age and ability range. This study was conducted in compliance with the Declaration of Helsinki. The protocol was peer-reviewed in the Department of Psychiatry at the University of Cape Town and submitted for ethical approval by the Faculty of Health Sciences, Human Research Ethics Committee (Ethics Ref 340/2015). Additional study sites obtained ethical approval from their respective HREC/IRB bodies.

5.2.2 Procedures

The TAND Checklist was administered to parents and caregivers of individuals

with TSC by the resident TSC coordinator and/or treating physician. The TAND Checklist follows the neuropsychiatric levels of investigation outlined previously (de Vries *et al.*, 2015a) and contains the following 12 sections: 1. Basic developmental milestones; 2. Current level of functioning; 3. Behavioural concerns; 4. Psychiatric disorders diagnosed; 5. Intellectual ability; 6. Academic skills; 7. Neuropsychological skills; 8. Psychosocial functioning; 9. Parent, caregiver, or self-rating of the impact of TAND; 10. Prioritisation list; 11. Additional concerns; and 12. Healthcare professional rating of the impact of TAND. Questions require simple YES or NO responses to most sections. For a detail about TAND and the TAND Checklist, including a downloadable version of the Checklist, please see de Vries *et al.* (2015a). A copy of the TAND Checklist is also provided in the appendix of the thesis.

5.2.3 Data Analysis

Statistical analysis was performed using a series of steps as outlined in **Figure 5.1**.

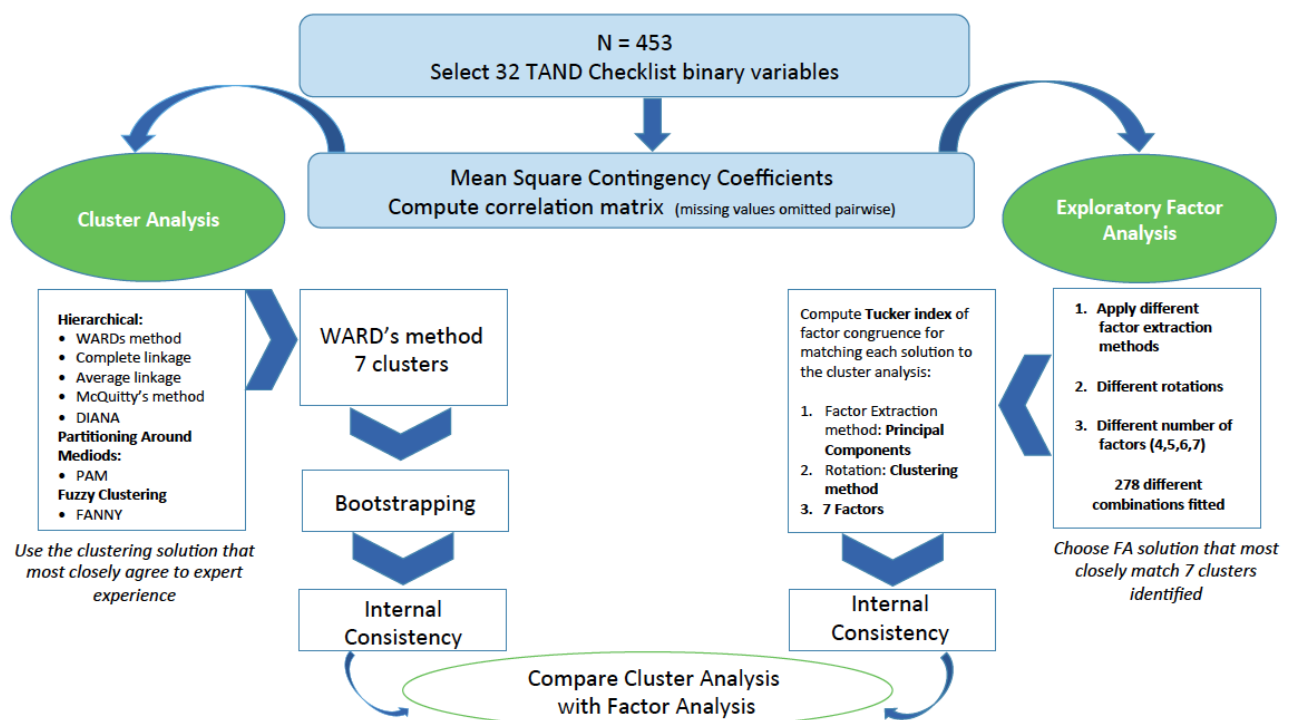


Figure 5.1. Steps followed during statistical analysis

STEP 1. Select TAND Checklist variables

The following sections of the TAND Checklist were included in the analysis: Section 3, behavioural challenges (19 questions/variables); Section 5, academic skills (4 variables); and Section 7, neuropsychological skills (6 variables). This equated to 29 dichotomous TAND variables. Sections 1 and 2 were omitted as they refer to developmental milestones (section 1) and current level of functioning (section 2). Section 4 addresses specific psychiatric diagnosis and section 6, Intellectual ability (3 options: normal ability, mild/moderate intellectual disability, severe/profound intellectual disability). These two sections were also excluded as they were not deemed appropriate in generating natural TAND clusters. Lastly, section 9 was also omitted as it refers to parent, caregiver, or self-rating of the impact of TAND (likert scale 1-10).

STEP 2. Compute Correlation Matrix

Mean squared contingency coefficient (Cramer, 1946) was used to compute a correlation matrix for the 29 binary variables selected from the TAND Checklist. Where missing values were present, these were omitted pairwise in correlation computations.

STEP 3. Cluster Analysis

Several clustering solutions were compared. Hierarchical clustering methods provide a clustering tree visually representing the merging of TAND variables and suggesting a suitable number of clusters. Complete linkage, average linkage, WARD's method and McQuitty's methods were applied with the `hclust()` R function. Although hierarchical clustering has often been used with great success, the algorithm is fairly naïve and some more recent methods in the R package `cluster` (R Core Team, 2016) were therefore also investigated. PAM (partitioning around medoids) is an extension of the popular k-means clustering method. K-means could not be applied given that it required a numerical data matrix rather than a dissimilarity matrix based on the square contingency coefficient correlation matrix. Given that most variables were binary (Yes/No), model-based clustering assuming an underlying Gaussian distribution was not considered suitable. The FANNY (fuzzy clustering) method applied to the data allocates a probability for belonging to each cluster rather than simply allocating each item to a single cluster. DIANA (divisive analysis), also a hierarchical clustering method, was also

used. In contrast to the other methods where larger clusters are formed by merging smaller clusters, this method forms smaller clusters by dividing larger clusters.

STEP 4. Bootstrapping

Following cluster analysis, a bootstrap method was applied to assess the statistical robustness or stability of the clustering solution. In a bootstrap analysis, the 'real world' analysis is repeated a large number, say 1000 times, in the 'bootstrap world'. Having 1000 bootstrap samples provide the researcher with an indication of the variability in the analysis, should it be repeated several times. For a single bootstrap replicate, a sample of size 453 patients is obtained through random sampling with replacement from the observed sample of 453 patients. This means that some patients can appear more than once in the bootstrap sample whilst others do not appear at all. Based on the bootstrap sample of patients, a square contingency coefficient correlation matrix was computed and hierarchical cluster analysis with WARD's method was performed. The number of clusters were fixed at 7 and a bootstrap replicate clustering solution was obtained. Repeating this process 1000 times, 1000 clustering solutions were obtained. In order to assess the robustness or stability of the original observed clustering solution, the number of times each pair of variables were clustered together was calculated. For a perfectly stable clustering solution with no random noise, two variables that were in the same cluster would have been expected to cluster together 1000 out of 1000 times (bootstrap value = 1) in the bootstrap replicates and two variables that belong to different clusters in the observed clustering solution would have clustered together 0 out of 1000 times (bootstrap value = 0) in the bootstrap replicate. In practice a stable solution would have items clustered together in the same cluster in the majority of bootstrap replicates and items not clustering together only occasionally in the same bootstrap cluster.

STEP 5. Exploratory Factor Analysis

After suitable clustering solutions were obtained and bootstrapping applied, exploratory factor analysis was employed with the function `fa()` from the R package `psych` (Revelle, 2016). The factor analysis was also performed on the mean squared contingency coefficient correlation matrix. All the different options of factor extraction and rotation available in the `fa()` R function were investigated.

These combinations were applied to solutions with between four and seven factors. In order to find the factor solution that best matched the cluster analysis solution, the Tucker index of factor congruence (Lorenzo-Seva and ten Berge, 2006) was used. A perfect match would have a square matrix with 1 on the diagonal (indicating a perfect match) and 0 on the off-diagonal (indicating a complete mismatch). Changing the order of the rows or columns of this matrix also represents a perfect match with the inconsequential naming of the clusters in a different order. To ensure a unique congruence matrix, the computed pairwise congruence matrix is optimally rotated to the indicator matrix. Rotation is based on Orthogonal Procrustes Analysis and the overall congruence summarised by the sum of the diagonal values. For cases other than 6 factors, the congruence matrix is padded with zeros to obtain a square matrix before rotation. For algebraic details, see **Appendix E**.

STEP 6. Test Internal Consistency

Reliability analysis was used to test the internal consistency of the TAND variables comprised in both the clusters identified and factors generated.

STEP 7. Compare Cluster Analysis findings with Exploratory Factor Analysis Results

Finally, we set out to compare the data-driven cluster solutions with the exploratory factor analysis in order to examine similarities and differences of the two approaches.

5.3 Results

5.3.1 Cluster Analysis

Hierarchical clustering with WARD's method produced a seven-cluster solution. A dendrogram of the WARD cluster analysis shows detail of the natural clustering of the TAND variables examined (**Figure 5.2**).

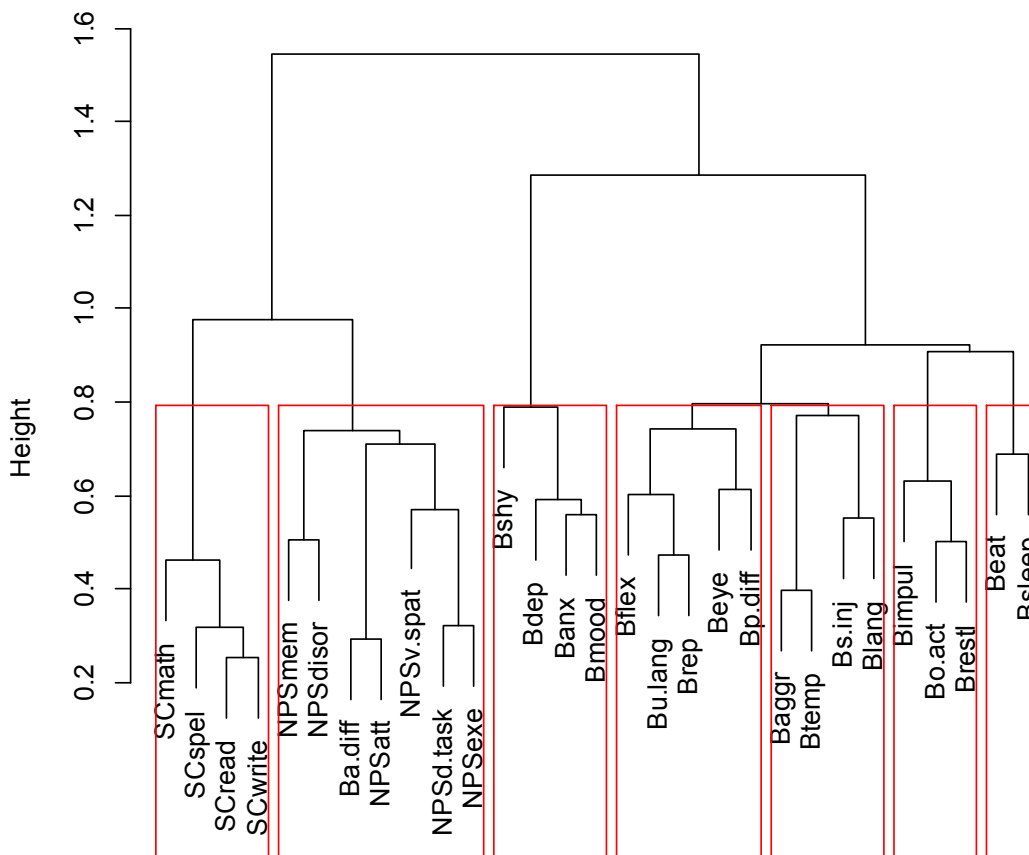


Figure 5.2. Dendrogram illustrating WARD's cluster analysis method which rendered 7 natural TAND Clusters (n= 453)

The first cluster included items on difficulties with mathematics, spelling, writing, and reading suggesting a natural 'Scholastic' cluster. The second cluster included items for memory difficulties, getting disorientated, attention difficulties at both a behavioural and neuropsychological level, difficulty with visuo-spatial tasks, dual-task difficulties, and executive skills difficulties. These suggested a natural 'Neuropsychological' cluster. The third cluster included extreme shyness, depressed mood, anxiety, and mood swings, suggesting a natural 'Mood/Anxiety' cluster. The fourth cluster grouped together inflexibility, unusual language (repeating words or phrases over and over again), repetitive behaviours, poor eye contact, and peer difficulties, suggesting Autism Spectrum Disorder (ASD)-like characteristics. The fifth cluster included aggressive outbursts, temper tantrums, self-injury, and absent or delayed onset of language. These characteristics were slightly mixed but overall supported a natural 'Behavioural Dysregulation' cluster. The sixth cluster, suggesting a natural 'Overactive/Impulsive' cluster contained impulsivity, over-activity, and restlessness. The seventh cluster contained two

biological items - difficulties with eating and sleep-related difficulties. We therefore refer to this as the 'Eat/Sleep' cluster.

5.3.2 Bootstrapping

Table 5.1 shows the bootstrapping results expressed as a proportion of times two items cluster together. The Scholastic cluster was very stable with all four items clustering together 100% of the time. A few other items showed bootstrapping values between 29% and 58% (Visuo-spatial skills, 58%; Dual-tasking, 29%; Executive Skills, 29%) in association with the scholastic items. All other items had bootstrap values < 20%. In the Neuropsychological cluster, items were stable with bootstrap values ranging from 100% (behavioural and neuropsychological attention difficulties; dual-tasking and executive skills), 92% (memory), 72% (disorientation), to 60% (visuo-spatial). Items in the Overactive/Impulsive cluster clustered together 100% of the time (overactive; restless) and 65% of the time included impulsivity. In the Mood/Anxiety cluster extreme shyness showed bootstrapping values between 42% and 91%; depressed mood between 49% and 91%; anxiety between 51% and 97%, and mood swings between 42% and 51%. The fifth cluster, Dysregulated Behaviour, showed two items (temper tantrums and aggressive outbursts) that clustered together 100% of the time and two items (self-injury and delayed language) that showed very low bootstrapping values with aggression/temper tantrums (19% and 30%). The ASD-like cluster items ranged between 26% and 97%. The items showed bootstrapping values between 24% and 65% (inflexibility 24%-48%, poor eye contact 35% and 65%, peer difficulty 31% - 65%) within this cluster. When considered alongside ASD-like cluster items, self-injury and delayed language clustered together 88% of the time and unusual language and repetitive behaviour 97%. These bootstrapping values were higher in the ASD-like cluster when compared with their bootstrapping scores obtained in the Dysregulated Behaviour cluster. The two items of the Eat/Sleep cluster, clustered together 78% of the time.

Table 5.1. Bootstrapping applied to WARD's cluster analysis, results expressed as a proportion of times two items cluster together

| | Scread | Scwrite | Scspel | Scmath | NPSv.spat | NPSd.task | NPSexe | NPSdisor | NPSmem | NPSatt | Ba.diff | Bimpul | Bo.act | Brestl | Bmood | Banx | Bdep | Bshy | Baggr | Btemp | Bp.diff | Beye | Bflex | Brep | Bu.lang | Blang | Bs.inj | Beat | Bsleep | |
|-----------|--------|---------|--------|--------|-----------|-----------|--------|----------|--------|--------|---------|--------|--------|--------|-------|------|------|------|-------|-------|---------|------|-------|------|---------|-------|--------|------|--------|------|
| SCread | 1.00 | 1.00 | 1.00 | 1.00 | 0.58 | 0.29 | 0.29 | 0.08 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.08 | 0.07 | 0.20 | 0.13 | 0.00 | 0.00 | |
| Scwrite | 1.00 | 1.00 | 1.00 | 1.00 | 0.58 | 0.29 | 0.29 | 0.08 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.08 | 0.07 | 0.20 | 0.13 | 0.00 | 0.00 | |
| Scspel | 1.00 | 1.00 | 1.00 | 1.00 | 0.58 | 0.29 | 0.29 | 0.08 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.08 | 0.07 | 0.20 | 0.13 | 0.00 | 0.00 | |
| SCmath | 1.00 | 1.00 | 1.00 | 1.00 | 0.58 | 0.29 | 0.29 | 0.08 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.07 | 0.06 | 0.20 | 0.13 | 0.00 | 0.00 | |
| NPSv.spat | 0.58 | 0.58 | 0.58 | 0.58 | 1.00 | 0.60 | 0.60 | 0.41 | 0.34 | 0.23 | 0.23 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.09 | 0.03 | 0.14 | 0.13 | 0.25 | 0.18 | 0.01 | 0.00 | |
| NPSd.task | 0.29 | 0.29 | 0.29 | 0.29 | 0.60 | 1.00 | 1.00 | 0.72 | 0.71 | 0.61 | 0.61 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.04 | 0.02 | 0.02 | 0.03 | 0.04 | 0.02 | 0.00 | 0.00 | |
| NPSexe | 0.29 | 0.29 | 0.29 | 0.29 | 0.60 | 1.00 | 1.00 | 0.72 | 0.71 | 0.61 | 0.61 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.04 | 0.02 | 0.02 | 0.03 | 0.04 | 0.02 | 0.00 | 0.00 | |
| NPSdisor | 0.08 | 0.08 | 0.08 | 0.08 | 0.41 | 0.72 | 0.72 | 1.00 | 0.92 | 0.64 | 0.64 | 0.08 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.05 | 0.03 | 0.03 | 0.03 | 0.04 | 0.03 | 0.00 | 0.01 | |
| NPSmem | 0.02 | 0.02 | 0.02 | 0.02 | 0.34 | 0.71 | 0.71 | 0.92 | 1.00 | 0.72 | 0.72 | 0.10 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | |
| NPSatt | 0.03 | 0.03 | 0.03 | 0.03 | 0.23 | 0.61 | 0.61 | 0.64 | 0.72 | 1.00 | 1.00 | 0.32 | 0.25 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.02 | 0.07 | 0.05 | 0.03 | 0.01 | 0.01 | 0.01 | 0.00 | 0.03 | 0.02 |
| Ba.diff | 0.03 | 0.03 | 0.03 | 0.03 | 0.23 | 0.61 | 0.61 | 0.64 | 0.72 | 1.00 | 1.00 | 0.32 | 0.25 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.02 | 0.07 | 0.05 | 0.03 | 0.01 | 0.01 | 0.01 | 0.00 | 0.03 | 0.02 |
| Bimpul | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.09 | 0.09 | 0.08 | 0.10 | 0.32 | 0.32 | 1.00 | 0.65 | 0.65 | 0.16 | 0.01 | 0.00 | 0.00 | 0.00 | 0.39 | 0.39 | 0.36 | 0.15 | 0.32 | 0.17 | 0.18 | 0.04 | 0.06 | 0.17 | 0.12 |
| Bo.act | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 | 0.25 | 0.25 | 0.65 | 1.00 | 1.00 | 0.09 | 0.00 | 0.00 | 0.00 | 0.24 | 0.24 | 0.14 | 0.07 | 0.13 | 0.11 | 0.11 | 0.06 | 0.08 | 0.09 | 0.07 | |
| Brestl | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 | 0.25 | 0.25 | 0.65 | 1.00 | 1.00 | 0.09 | 0.00 | 0.00 | 0.00 | 0.24 | 0.24 | 0.14 | 0.07 | 0.13 | 0.11 | 0.11 | 0.06 | 0.08 | 0.10 | 0.07 | |
| Bmood | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.09 | 0.09 | 1.00 | 0.51 | 0.49 | 0.42 | 0.52 | 0.52 | 0.21 | 0.09 | 0.29 | 0.10 | 0.11 | 0.03 | 0.07 | 0.13 | 0.21 | |
| Banx | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.51 | 1.00 | 0.97 | 0.88 | 0.04 | 0.03 | 0.03 | 0.01 | 0.25 | 0.01 | 0.01 | 0.00 | 0.00 | 0.02 | 0.19 | |
| Bdep | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.49 | 0.97 | 1.00 | 0.91 | 0.02 | 0.01 | 0.02 | 0.00 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.18 | |
| Bshy | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.42 | 0.88 | 0.91 | 1.00 | 0.01 | 0.01 | 0.03 | 0.02 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.17 | |
| Baggr | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.02 | 0.39 | 0.24 | 0.24 | 0.52 | 0.04 | 0.02 | 0.01 | 1.00 | 1.00 | 0.45 | 0.26 | 0.44 | 0.30 | 0.32 | 0.19 | 0.30 | 0.22 | 0.16 | |
| Btemp | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.02 | 0.39 | 0.24 | 0.24 | 0.52 | 0.03 | 0.01 | 0.01 | 1.00 | 1.00 | 0.46 | 0.26 | 0.43 | 0.30 | 0.32 | 0.19 | 0.30 | 0.22 | 0.16 | |
| Bp.diff | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.01 | 0.01 | 0.01 | 0.02 | 0.07 | 0.07 | 0.36 | 0.14 | 0.14 | 0.21 | 0.03 | 0.02 | 0.03 | 0.45 | 0.46 | 1.00 | 0.65 | 0.48 | 0.42 | 0.43 | 0.31 | 0.33 | 0.47 | 0.34 | |
| Beye | 0.03 | 0.03 | 0.03 | 0.03 | 0.09 | 0.04 | 0.04 | 0.05 | 0.04 | 0.05 | 0.05 | 0.15 | 0.07 | 0.07 | 0.09 | 0.01 | 0.00 | 0.02 | 0.26 | 0.26 | 0.65 | 1.00 | 0.35 | 0.58 | 0.57 | 0.59 | 0.55 | 0.38 | 0.22 | |
| Bflex | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.02 | 0.02 | 0.03 | 0.02 | 0.03 | 0.03 | 0.32 | 0.13 | 0.13 | 0.29 | 0.25 | 0.22 | 0.19 | 0.44 | 0.43 | 0.48 | 0.35 | 1.00 | 0.54 | 0.56 | 0.24 | 0.26 | 0.18 | 0.15 | |
| Brep | 0.08 | 0.08 | 0.08 | 0.07 | 0.14 | 0.02 | 0.02 | 0.03 | 0.01 | 0.01 | 0.01 | 0.17 | 0.11 | 0.11 | 0.10 | 0.01 | 0.00 | 0.00 | 0.30 | 0.30 | 0.42 | 0.58 | 0.54 | 1.00 | 0.97 | 0.65 | 0.64 | 0.14 | 0.05 | |
| Bu.lang | 0.07 | 0.07 | 0.07 | 0.06 | 0.13 | 0.03 | 0.03 | 0.03 | 0.01 | 0.01 | 0.01 | 0.18 | 0.11 | 0.11 | 0.11 | 0.01 | 0.00 | 0.00 | 0.32 | 0.32 | 0.43 | 0.57 | 0.56 | 0.97 | 1.00 | 0.62 | 0.62 | 0.14 | 0.05 | |
| Blang | 0.20 | 0.20 | 0.20 | 0.20 | 0.25 | 0.04 | 0.04 | 0.04 | 0.01 | 0.01 | 0.01 | 0.04 | 0.06 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | 0.19 | 0.19 | 0.31 | 0.59 | 0.24 | 0.65 | 0.62 | 1.00 | 0.88 | 0.19 | 0.07 | |
| Bs.inj | 0.13 | 0.13 | 0.13 | 0.13 | 0.18 | 0.02 | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.06 | 0.08 | 0.08 | 0.07 | 0.00 | 0.00 | 0.00 | 0.30 | 0.30 | 0.33 | 0.55 | 0.26 | 0.64 | 0.62 | 0.88 | 1.00 | 0.20 | 0.08 | |
| Beat | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.03 | 0.03 | 0.17 | 0.09 | 0.10 | 0.22 | 0.13 | 0.02 | 0.01 | 0.01 | 0.22 | 0.22 | 0.47 | 0.38 | 0.18 | 0.14 | 0.14 | 0.19 | 0.20 | 1.00 | 0.78 | |
| Bsleep | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.02 | 0.12 | 0.07 | 0.07 | 0.21 | 0.19 | 0.18 | 0.17 | 0.16 | 0.16 | 0.34 | 0.22 | 0.15 | 0.05 | 0.05 | 0.07 | 0.08 | 0.78 | 1.00 | |

5.3.3 Factor Analysis

Factor loadings (cut-off >0.35) from exploratory factor analysis are shown in **Table 5.2**. The factor analysis solution which most closely matched WARD's hierarchical cluster analysis was the principal components factor extraction method with clustering method rotation. Results supported a seven-factor solution which was, on the whole, very similar to the cluster solution outlined above. Three items cross-loaded onto more than one factor. These were self-injurious behaviour (ASD-like factor and Eat/Sleep factor), sleeping difficulties (Eat/Sleep factor and Mood/Anxiety factor), and disorientation (ASD-like factor and the Neuropsychological factor).

Table 5.2. Factor analysis results identifying a seven-factor solution (cut-off >0.35)

| | PA1 | PA2 | PA5 | PA3 | PA4 | PA6 | PA7 |
|-----------|-------|-------|-------|-------|-------|-------|-------|
| SCread | 0.93 | 0.00 | -0.03 | 0.01 | 0.00 | -0.07 | -0.04 |
| SCwrite | 0.77 | -0.07 | 0.10 | 0.00 | 0.10 | -0.04 | 0.01 |
| SCspel | 0.76 | -0.04 | 0.00 | 0.06 | -0.06 | 0.03 | 0.07 |
| SCmath | 0.60 | 0.04 | 0.10 | 0.06 | -0.22 | 0.13 | 0.07 |
| Banx | 0.00 | 0.71 | 0.03 | 0.01 | -0.08 | -0.03 | 0.11 |
| Bdep | -0.15 | 0.71 | -0.07 | 0.12 | -0.15 | -0.03 | 0.04 |
| Bshy | -0.01 | 0.41 | -0.05 | 0.04 | -0.06 | -0.04 | 0.16 |
| Bmood | 0.01 | 0.51 | -0.06 | -0.03 | 0.04 | 0.30 | 0.05 |
| Bflex | 0.17 | 0.46 | 0.12 | 0.02 | 0.15 | -0.01 | -0.03 |
| Bu.lang | 0.10 | 0.20 | 0.36 | -0.01 | 0.16 | 0.04 | -0.04 |
| Beye | -0.11 | 0.12 | 0.37 | 0.17 | -0.01 | 0.02 | 0.20 |
| Bp.diff | -0.12 | 0.23 | 0.18 | 0.14 | 0.00 | 0.14 | 0.27 |
| Brep | 0.11 | 0.16 | 0.64 | -0.17 | 0.26 | -0.13 | 0.09 |
| Blang | 0.07 | -0.20 | 0.55 | 0.02 | -0.05 | 0.04 | 0.31 |
| Bs.inj | 0.05 | 0.03 | 0.38 | -0.19 | -0.05 | 0.21 | 0.39 |
| Baggr | -0.01 | 0.23 | -0.02 | -0.01 | 0.06 | 0.65 | -0.01 |
| Btemp | 0.04 | 0.02 | -0.06 | -0.07 | 0.12 | 0.74 | 0.04 |
| Ba.diff | 0.15 | 0.03 | -0.19 | 0.56 | 0.27 | -0.06 | 0.13 |
| NPSmem | -0.16 | 0.13 | 0.05 | 0.63 | 0.07 | -0.07 | -0.11 |
| NPSatt | 0.00 | -0.05 | -0.18 | 0.81 | 0.15 | 0.01 | 0.13 |
| NPSd.task | 0.24 | 0.08 | 0.03 | 0.64 | -0.08 | 0.06 | -0.16 |
| NPSexe | 0.03 | 0.06 | 0.25 | 0.68 | -0.06 | 0.02 | -0.18 |
| NPSdisor | -0.12 | 0.03 | 0.40 | 0.50 | 0.03 | -0.06 | -0.11 |
| NPSv.spat | 0.03 | -0.15 | 0.59 | 0.33 | -0.17 | -0.07 | 0.05 |
| Bo.act | -0.18 | -0.11 | 0.15 | 0.11 | 0.73 | 0.13 | -0.17 |
| Brestl | -0.10 | -0.02 | 0.14 | 0.02 | 0.62 | -0.10 | 0.18 |
| Bimpul | 0.08 | 0.04 | -0.12 | 0.22 | 0.43 | 0.18 | -0.06 |
| Beat | 0.09 | 0.15 | 0.13 | -0.05 | 0.05 | -0.06 | 0.44 |
| Bsleep | -0.01 | 0.36 | 0.09 | -0.04 | -0.04 | -0.12 | 0.45 |

5.3.4 Internal Consistency

Using Chronbach's alpha to evaluate internal consistency in the cluster analysis solutions, 5 clusters scored > 0.7 indicating good to excellent internal consistency (see **Table 5.3**): Scholastic (0.97), Neuropsychological (0.87), ASD-like (0.76), Behavioural Dysregulation (0.74), and Overactive/Impulsive (0.70). The remaining 2 clusters had lower alpha values: Mood/Anxiety (0.69) and Eat/Sleep (0.48). In the factor analysis solutions 6 factors showed good to excellent internal consistency (see **Table 5.4**): Scholastic (0.97), Neuropsychological (0.86), ASD-like (0.79), Behavioural Dysregulation (0.75), and Overactive/Impulsive (0.70). Only the Eat/Sleep factor scored <0.7 with an alpha = 0.54, suggesting poor internal consistency.

Table 5.3. Descriptive statistics for the seven natural TAND clusters identified through WARD's method. Cronbach alpha \geq 0.7 indicates good internal consistency

| Cluster | No. of Items | Alpha |
|---------------------------|--------------|-------|
| Scholastic | 4 | 0.97 |
| Neuropsychological | 7 | 0.87 |
| Mood/Anxiety | 4 | 0.69 |
| ASD-Like | 5 | 0.76 |
| Behavioural Dysregulation | 4 | 0.74 |
| Hyperactive/Impulsive | 3 | 0.70 |
| Eat/Sleep | 2 | 0.48 |

Table 5.4. Descriptive statistics for the seven TAND Factors identified through Exploratory Factor Analysis. Cronbach alpha \geq 0.7 indicates good internal consistency.

| Cluster | No. of Items | Alpha |
|---------------------------|--------------|-------|
| Scholastic | 4 | 0.97 |
| Mood/Anxiety | 6 | 0.74 |
| ASD-like | 7 | 0.79 |
| Behavioural Dysregulation | 2 | 0.75 |
| Neuropsychological | 6 | 0.86 |
| Overactive/Impulsive | 3 | 0.70 |
| Eat/Sleep | 3 | 0.54 |

5.3.5 Comparison of cluster analysis and factor analysis findings

Factor analysis confirmed a similar profile found in cluster analysis, but with some slight variance between clusters and factors (**Figure 5.3**). Cluster and factor analysis showed the ‘Overactive/Impulsive’ and ‘Scholastic’ clusters to be clearly distinct, the ‘Mood/Anxiety’ and ‘Neuropsychological’ clusters to be fairly distinct, while the other three clusters/factors showed more evidence of cross-loading between items in different clusters/factors.

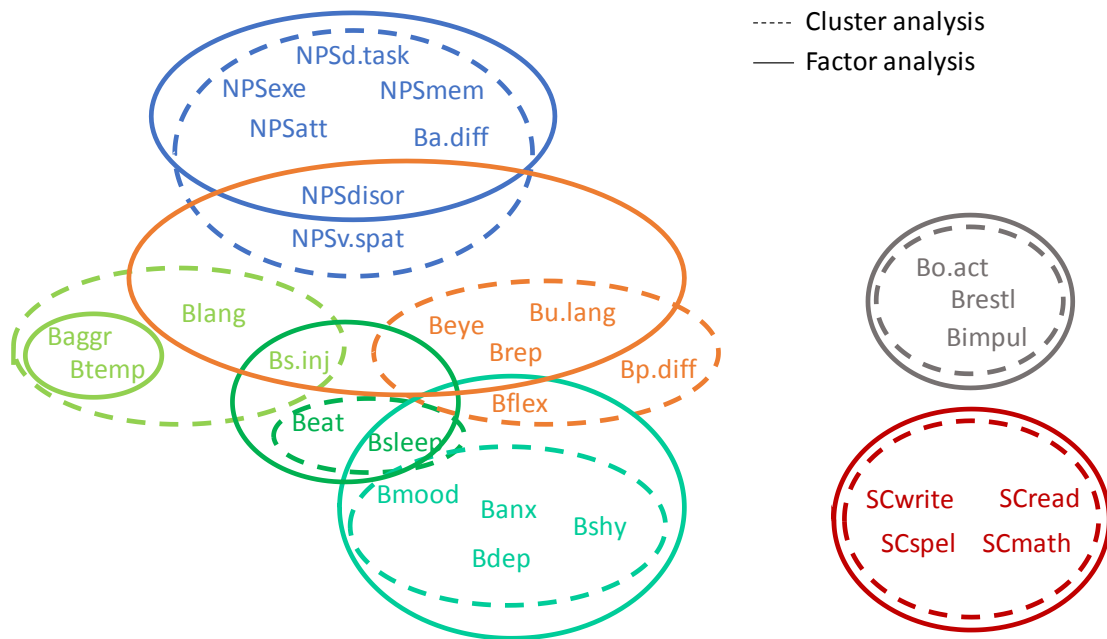


Figure 5.3. Relationship between natural TAND Clusters and Factor Analysis. Results show two distinct clusters (Scholastic and Overactive/Impulsive), three fairly distinct (Mood/Anxiety, Neuropsychological, Eat/Sleep), and significant cross-loading between the other two clusters (ASD-like and Behavioural Dysregulation).

5.4 Discussion

TSC-Associated Neuropsychiatric Disorders (TAND) have largely gone undiagnosed and untreated despite affecting nine out of ten individuals with TSC. This is mainly due to lack of awareness, apparent unique TAND profiles, and a lack of knowledge and experience in treating TAND. We proposed that the identification of naturally occurring TAND clusters may improve assessment and intervention. In a pilot feasibility we showed that a data-driven approach may be able to identify natural clusters of TAND (Leclezio *et al.*, 2017). However, despite the potentially interesting

suggestion, findings required larger-scale replication and extension, particularly to evaluate the robustness of proposed natural TAND clusters. In this study various cluster analysis techniques and exploratory factor analysis was applied in a large and diverse international sample (n= 453). In addition, bootstrapping and internal consistency analyses were performed.

Results identified seven natural TAND clusters, and bootstrapping showed 5 of the 7 clusters to be statistically robust. The two apparently less robust clusters were the 'Dysregulated Behaviour' and 'ASD-like' clusters. Two of the items clustered with Dysregulated Behaviours (self-injury and language delay) showed stronger association with the ASD-like cluster using bootstrapping, suggesting that these may more appropriately be linked to the ASD-like cluster.

Exploratory factor analysis showed that a 7-factor solution mapped well onto the majority of clusters, but with the greatest cross-loading between the ASD-like, Dysregulated Behaviour and Mood/Anxiety clusters. The Scholastic, Neuropsychological, Overactive/Impulsive, and Eat/Sleep clusters otherwise showed good agreement between clusters and factors.

Internal consistency for clusters and factors was good to excellent internal for 5/7 of the clusters generated (except for the Mood/Anxiety and Eat/Sleep clusters) and 6/7 of the factors identified (except for the Eat/Sleep factor).

In slight contrast to the initial pilot feasibility study (Leclezio *et al.*, 2017, under review) which suggested 6 natural TAND clusters, this larger-scale study identified 7 TAND clusters. In this study, the two biological or vegetative items (sleeping/eating) grouped together, whereas in the pilot study they were incorporated into the ASD-like cluster (difficulties with eating) and the Mood/Anxiety cluster (sleep difficulties). The remaining 6 clusters, however, were remarkably similar to the findings from the pilot study, reproducing the same clusters, with a few items cross-loading and/or represented elsewhere.

5.4.1 Proposed Natural TAND Clusters

Taking together all results, we propose the following 7 natural TAND clusters for future use in clinical practice and research (see **Table 5.5**).

1. Scholastic cluster

The first of the seven natural TAND clusters identified is a 'Scholastic' cluster indicating difficulties relating to reading, writing, spelling and mathematics. The items in the Scholastic cluster (rendered by both cluster analysis and factor analysis) showed perfect bootstrapping, very high factor loadings and alpha scores, indicating the close relationship and reliability between items. Findings highlight the need for assessment in this cluster if an individual shows signs of difficulty across any one of the four items. Academic difficulties are a common concern in TSC (Joinson *et al.*, 2003; de Vries, 2010a; Kingswood *et al.*, 2017) and not only affects school-aged children, but also have long-term consequences in adulthood.

2. Overactive/Impulsive cluster

Both cluster analysis and factor analysis included overactivity, restlessness, and impulsivity in this cluster. Bootstrapping and internal consistency were very good, indicative of the reliability of items and how they group together. This cluster appears very meaningful given the high rates of ADHD reported in TSC (Prather and de Vries, 2004; de Vries, 2010a). However, it is of interest that the cluster did not include attentional difficulties, which were grouped in the neuropsychological cluster. This may suggest ADHD in TSC to be more typically of the 'predominantly hyperactive/impulsive subtype', or could suggest that there may be differential pathways to the attentional and hyperactive/impulsive deficits seen in TSC.

3. Neuropsychological cluster

This cluster includes memory deficits, disorientation, neuropsychological attention deficits as well as attention deficits in daily life, dual task deficits, executive deficits, and visuo-spatial deficits. Whilst visuo-spatial deficits was grouped within the ASD factor, cluster analysis grouped visuo-spatial deficits with the other neuropsychological skills. Bootstrapping supported the clustering with neuropsychological skills, but confirmed a frequent co-occurrence with the scholastic cluster. Taking all data together, we propose to group visuospatial skills within the neuropsychological cluster, a recommendation we believe also makes clinical sense. Based on the existing TSC literature, the cluster maps very well onto the high rates of a range of neuropsychological attentional, executive, and memory deficits reported (Ridler *et al.*, 2007; de Vries, *et al.*, 2009; de Vries, 2010a; Tierney *et al.*, 2011).

4. Mood/Anxiety cluster

Four items are proposed in this cluster – anxiety, depressed mood, mood swings and extreme shyness. We observed that factor analysis included inflexibility and sleep-related problems with the four other items. However, bootstrapping classified these two items in the mood/anxiety cluster only 19% - 29% of the time. Given the cluster analysis and bootstrapping observed, we propose to group inflexibility with ASD-like features, and ‘sleep difficulties’ with the Eat/Sleep cluster. The remaining four items (mood swings, anxiety, depressed mood, extreme shyness) are often seen in children and adults with TSC (Lewis *et al.*, 2004; de Vries *et al.*, 2007; de Vries, 2010a; Kingswood *et al.*, 2017).

5. Behavioural Dysregulation cluster

The behavioural dysregulation cluster includes aggressive outbursts and temper tantrums. Cluster analysis included self-injurious behaviour and delayed language also in the cluster. However, bootstrapping did not support the robustness of these items in the cluster. Instead, both bootstrapping and factor analysis showed stronger support for self-injury and delayed/absent language to be part of the ASD-like cluster. One of the biggest concerns to families is the high rate of ‘behaviours that challenge’ seen in TSC specifically with regards to aggression and temper tantrums, self-injury and damage to property (Prather and de Vries, 2004; de Vries *et al.*, 2007; de Vries, 2010a; Eden *et al.*, 2016; Wilde *et al.*, 2017). It was therefore of interest that a specific and distinct cluster of dysregulated behaviours was identified here.

6. ASD-like cluster

This natural TAND cluster includes 7 items - inflexibility, unusual language, repetitive behaviour, poor eye contact, peer difficulty, self-injury, and delayed/absent language. As outlined above, initial cluster analysis did not include ‘self-injury’ and ‘delayed language’ in the ASD-like cluster, but bootstrapping and factor analysis suggested these characteristics to be more likely to co-occur with ASD-like rather than with other TAND behaviours. TSC is one of the medical conditions most strongly associated with ASD (Bolton *et al.*, 2002). It was therefore of interest to see the natural emergence of an ASD-like cluster of behaviours (Bolton *et al.*, 2002; Prather and de Vries, 2004; de Vries, 2010a). From a clinical perspective, the strong association between self-injury and delayed language maps well onto other studies in

TSC (Eden *et al*, 2014; Wild *et al.*, 2017). Even though we proposed placing self-injury in the ASD-like cluster there is clear cross-loading also with the ‘Sleep/Eat’ cluster reinforcing the finding by Wilde and others that self-injury can be associated with a range of manifestations.

7. Eat/Sleep cluster

This cluster includes eating and sleep difficulties. Sleeping and eating are such fundamental biological or vegetative functions that it was not surprising to see them cluster together. This cluster was not identified in the pilot study, but this much larger sample suggested that these concerns group together reliably. Importantly, both sleep and eating difficulties cross-load with other clusters, underlining the fact that they often co-occur with other neuropsychiatric difficulties. However, the fact that they cluster independently suggests the need to investigate these in their own right, not only in the context of other so-called co-morbid conditions.

Table 5.5. Proposed seven natural TAND clusters with items contained in each cluster; and Chronbach’s alpha scores per cluster

| Cluster | No. of Items | Items | Alpha |
|----------------------|--------------|--|-------|
| Scholastic | 4 | <ul style="list-style-type: none"> • Reading • Writing • Spelling • Mathematics | 0.97 |
| Overactive/Impulsive | 3 | <ul style="list-style-type: none"> • Overactive • Impulsive • Restless | 0.70 |
| Neuropsychological | 7 | <ul style="list-style-type: none"> • Memory • Disorientation • Attention difficulties (behaviour) • Attention difficulties (neuropsychological) • Visuo-spatial • Dual-tasking • Executive skills | 0.87 |
| Mood/Anxiety | 4 | <ul style="list-style-type: none"> • Mood swings • Anxiety • Depressed mood | 0.69 |

| | | | |
|---------------------------|---|--|------|
| | | <ul style="list-style-type: none"> • Extreme shyness | |
| Behavioural Dysregulation | 2 | <ul style="list-style-type: none"> • Aggressive outbursts • Temper tantrums | 0.75 |
| ASD-Like | 7 | <ul style="list-style-type: none"> • Inflexible • Unusual language • Repetitive behaviour • Poor eye contact • Peer difficulties • Self-injury • Delayed language | 0.80 |
| Eat/Sleep | 2 | <ul style="list-style-type: none"> • Eat • Sleep | 0.48 |

* Cronbach alpha \geq 0.7 indicates internal consistency

5.4.2 Limitations

Firstly, the TAND Checklist was used here to identify potential natural TAND clusters and no other sources of information that may be relevant in cluster analysis or factor analysis, such as clinical evaluations or neuropsychological assessments were included. We acknowledge that it is therefore theoretically possible that other clusters and factors may be identified using different kinds of multi-level data. However, we specifically wanted to use the TAND Checklist for this purpose, given that it is a simple, yet systematic, and freely available tool that could easily be implemented in real life settings around the globe. As reported earlier, pilot validation of the TAND Checklist (Leclezio *et al.*, 2015) indicated that the TAND Checklist was a valid tool in extrapolating multi-level neuropsychiatric manifestations in TSC. Secondly, we acknowledged that all the data were ‘lifetime’ data. We have therefore not been able to examine the developmental pattern of natural TAND clusters, which may have a more dynamic nature than captured in our work. However, we hope that our findings will set the scene to examine longitudinal aspects of natural TAND clusters in future large-scale studies. Thirdly, the current TAND Checklist collects data in a dichotomous fashion. The study was therefore not able to explore the subtleties of severity that may be important to examine in natural TAND clusters in future.

5.5 Conclusion

This study set out to replicate and expand the potential of identifying natural TAND clusters from a large range of apparently independent neuropsychiatric features in individuals with TSC. Based on the largest collection of TAND Checklist data to date, our analyses generated seven natural TAND clusters that were statistically robust, and seemed to have good clinical face validity.

We therefore propose the identification of these clusters to be a useful first step as a guide to further assessment and treatment options in clinical practice. Next steps could include the development of targeted teaching and training to professionals and individuals with TSC around the seven natural TAND clusters, and identification of appropriate evidence-based resources and interventions that map onto these clusters. At a scientific level, we propose that identification of these naturally-occurring clusterings of the neuropsychiatric phenotype of TSC may be the first step towards a more dimensional, data-driven approach to the study of the aetiology and treatments (molecular and otherwise) of individuals with TSC.

5.6 Chapter Summary

Tuberous Sclerosis Complex (TSC) is associated with a wide range of behavioural, psychiatric, intellectual, learning, neuropsychological and psychosocial difficulties. The lifetime prevalence of these TSC-Associated Neuropsychiatric Disorders (TAND) is in the region of 90%. Each individual appears to present with their very own unique TAND profile, posing significant challenges for diagnosis, psycho-education, and intervention planning. Identifying natural TAND clusters will significantly improve our ability to identify and treat TAND. A recent pilot study showed that a combination of cluster analysis and factor analysis methods may be able to identify clinically-meaningful natural TAND clusters. In this study we set out to confirm and expand these findings in a larger dataset. TAND Checklist data were collected from 453 individuals across 6 international sites with a confirmed clinical diagnosis of TSC. Using R, the open-source statistical platform, mean squared contingency coefficients were calculated to produce a correlation matrix, and various cluster analyses and exploratory factor analysis (EFA) were examined. To examine the statistical robustness of clusters rendered, bootstrapping was applied. Thereafter Chronbach's

alpha was calculated to measure internal consistency of clusters and factors. WARD's method rendered seven natural TAND clusters with good robustness on bootstrapping. Cluster analysis showed significant convergence with an exploratory factor analysis solution, and, with the exception of one cluster, the internal consistency of the emerging clusters were good to excellent. Taking together all findings, seven natural TAND clusters with good clinical face validity are proposed. These include a 'Scholastic' cluster, an 'Overactive/Impulsive' cluster, a 'Neuropsychological' cluster, a 'Mood/Anxiety' cluster, a 'Behavioural Dysregulation' cluster, an, an 'ASD-like' cluster, and an 'Eat/Sleep' cluster. In this study, we used a data-driven computational approach and identified seven natural TAND clusters from within highly variable TAND Checklist data. The larger-scale study findings were remarkably consistent with pilot findings, supporting the robustness of these naturally occurring clusters. We propose that these seven natural TAND clusters could be used to develop novel approaches to identification and treatment of TAND. Our results suggest that these natural TAND clusters may have differential aetiological underpinnings and responses to molecular and other treatments.

CONCLUSIONS

6.1 Introduction

TSC-Associated Neuropsychiatric Disorders (TAND) comprise some of the greatest concerns to families and individuals with TSC, contributing very significantly to the burden of disease in TSC. Unfortunately, these concerns have gone largely undiagnosed and untreated due to lack of awareness, knowledge and resources for TAND. In order to raise awareness of these difficulties, the term TAND was coined and a TAND Checklist developed to aid clinicians in the systematic review of the range of neuropsychiatric manifestations seen. However, another obstacle to the assessment and treatment of TAND has been the perceived overwhelming uniqueness of TAND profiles observed. To many clinicians around the globe, the vast range and different levels of potential difficulties, often co-occurring and changing with time, simply left them unable to know where to start and what to do. In this thesis, the general aim was to identify natural TAND clusters, with the objective of reducing this perceived overwhelming uniqueness of individual TAND profiles. The specific aims included an overview of the neuropsychiatric phenotype observed in TSC, a reflection on the current challenges faced when striving to address TAND in both a clinical setting and from a research perspective, a pilot feasibility study to identify appropriate data-reduction techniques that may be able to generate natural TAND clusters, and finally a larger-scale study to identify natural TAND clusters proposed to be used by clinicians in the assessment and treatment of TAND, and by researchers in their quest for a better understanding of the aetiology and mechanisms underlying TAND.

6.2 Thesis Overview and Conclusions

In chapter 1, we outlined the thesis and presented a review of the physical characteristics and molecular understanding of TSC. In chapter 2, we reviewed the neuropsychiatric phenotype observed in TSC with a focus on recent advances of relevance to psychiatry, neuropsychiatry and the mental health of individuals with TSC. Chapter 3 hypothesised that natural TAND clusters may exist and proposed that the reduction of complexity seen in TAND could be achieved by searching for such clusters. In chapter 4, the pilot feasibility study was presented, which, using a modest sample, identified suitable methods for cluster and factor analysis. Using these methods, six potential natural TAND clusters were identified. Results therefore suggested that it may indeed be possible to use these techniques to generate natural clusters with good clinical face validity. In chapter 5, the main data chapter of the thesis, we applied the data-reduction techniques identified in the pilot study to a significantly larger sample for the identification of natural TAND clusters. Combining cluster analysis, bootstrapping, factor analysis and calculation of internal consistency across clusters and factors, we proposed seven natural TAND clusters. These were a Scholastic cluster, a Neuropsychological cluster, an Overactive/Impulsive cluster, a Dysregulated Behaviour cluster, a Mood/Anxiety cluster, an ASD-like cluster, and an Eat/Sleep cluster.

6.3 Implication of this research

6.3.1 Educating a wide range of healthcare and other professionals

One of the obstacles identified in this thesis refers to treatment paralysis i.e. the fact that many professionals, including mental health professionals, do not feel as though they have enough knowledge/expertise to treat neuropsychiatric manifestations observed in TSC, despite being the treating team. With the identification of natural TAND clusters, one can now customise and develop training materials based on the characteristics of each cluster. The development of a TAND curriculum, guided by these seven clusters, may prove to be very helpful in educating a range of professionals and clinicians, from support workers, healthcare workers, to nurses, therapists and physicians. The

hope is that empowering a wide range of healthcare and other professionals, should ultimately address the lack of assessment and treatment of TAND currently seen in the TSC community.

6.3.2 Educating parents, carers and individuals with TSC

We propose that the seven natural TAND clusters can also become the basis for education of parents, carers and individuals with TSC as a strategy to empower them to be able to do early identification of 'red flags' and concerns about TAND, to know what they can do to manage aspects of TAND, and to know how and when to seek additional professional support, assessment or treatment.

6.3.3. Linking resources to newly proposed natural TAND clusters

In support of the educational potential of the natural TAND clusters outlined above, the development of a TAND toolkit consisting of tools, tried and tested interventions and local resources to guide clinicians and families, will complement any educational strategies. We propose that the identified natural TAND clusters could play a critical role in the development of such a toolkit. These seven clusters may be used to organise resources into groups (clusters) that make clinical sense, pre-empt potential other difficulties associated with the specific cluster, and ensure a comprehensive inclusion of interventions.

6.3.4 New directions in the aetiology and molecular treatment of TAND

Traditionally, treatment options for TAND have been the same as standard of care for a typical diagnosis of ADHD, for example. However, with the advent of molecular targeted treatment options in TSC (specifically mTOR inhibitors), there has been great interest in the potential of mTOR inhibitors to modulate or treat neuropsychiatric manifestations. In a recent study by Krueger and colleagues (2017) the team investigated the effect of mTOR inhibitors on various aspects of TAND, including a range of neuropsychological skills, academic/scholastic skills and ASD-like characteristics. The results, based on a randomised-controlled trial of about 50 participants, did not seem to identify any obvious signals of change in relation to mTOR inhibition treatment. In a recent presentation, prior to the release of the Krueger *et al* findings (Leclezio and de Vries, 2016b), we suggested that there may be many different reasons why

clinical trials of TAND may be difficult. Using data from the TESTALL trial (Davies *et al.*, 2011), we showed that factors include the challenge of differing baseline scores between participants, challenges in the specific assessment tools used, timing of assessment during trial and at completion, duration of treatment, and potentially highly variable individual treatment responses (Leclezio and de Vries, 2016b). The identification of the seven natural TAND clusters in this thesis opens up the possibility that different natural TAND clusters may have differential responses to mTOR inhibitor treatments. This, in turn, may suggest that different clusters may be subserved by different molecular mechanisms. Utilisation of the newly identified natural TAND clusters may therefore inform the development of novel trial design and outcome measures. Apart from molecular mechanisms, we propose that different natural TAND clusters may also have differential structural brain correlates, and genotype-phenotype correlations.

6.4. Limitations of this study

- 6.4.1 During these studies, the TAND Checklist was the only tool used to identify potential natural TAND clusters. Therefore, there are no additional sources of information that may be relevant in cluster analysis. We therefore acknowledge that data-reduction approaches using different types of data may render different natural TAND clusters. However, pilot validation of the TAND Checklist (Leclezio *et al.*, 2015) indicated that the TAND Checklist was comprehensive and valid tool in extrapolating multilevel neuropsychiatric manifestations observed in TSC. In addition, we specifically wanted to explore clustering and data reduction using the TAND Checklist, in the hope that this may increase the clinical usefulness and global reach of the Checklist.
- 6.4.2 The sample used in the study was derived from a number of international TSC clinics, which may not have been completely representative of the TSC population as a whole. One might expect clinical data to be skewed towards the more severe manifestations of TSC. However, the deliberate attempt to get a larger-scale diverse sample of individuals with TSC from

six clinics around the globe, aimed to increase the representativeness of the sample. An ideal next step would be to evaluate these findings in an epidemiological TSC sample.

6.4.3 We only used the lifetime TAND Checklist, and data were therefore cross-sectional in nature. Given the age-related expression and manifestation of physical and neuropsychiatric manifestations in TSC, it would be of great interest to examine the emergence and development of TAND in a longitudinal cohort.

6.5. Future directions

6.5.1 With the application of factor analysis, it is possible to develop a method to generate a profile for each individual with TSC that indicates which clusters are involved, and to quantify the extent to which they are affected in that cluster. Such information could be clinically very useful to identify and recommend interventions and resources that may benefit the individual with TSC and their family in a personalised and targeted way. In addition, these clusters and scores may also have direct utility as a patient-reported outcome measure of TAND in pharmacological and non-pharmacological clinical trials and in the context of service evaluation.

6.5.2 The development of a TAND toolkit will be beneficial in directing clinicians and families to relevant resources, such as good practice guidelines, evidence-based interventions and local resources for support.

6.5.3 TSC stakeholders very strongly endorse the need for a patient-reported, accessible version of the TAND Checklist that could identify TAND profiles. Very consistently professional and family stakeholders recommend the use of technology, specifically the development of a TAND Checklist App as preferred mechanism to achieve this goal. Development and validation of a smartphone application (TAND-APP) as potential self/patient-reported outcome measure for TAND profiles and the burden of TAND, could therefore be a very powerful clinical application of the findings from this thesis.

6.5.4 Using the natural TAND clusters and a quantification of the TAND

Checklist could be very helpful in the scientific study of TAND, from longitudinal descriptive studies of the emergence of TAND, to correlational studies such as examination of functional and structural contributions to TAND, to experimental studies such as examination of the differential response to mTOR inhibition of TAND.

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Appendix A: TAND Checklist

THE TAND CHECKLIST *Lifetime version (TAND-L)*

Tuberous Sclerosis Complex (TSC) is associated with a range of neuropsychiatric disorders which we refer to as **TAND (TSC-Associated-Neuropsychiatric-Disorders)**. All people with TSC are at risk of having some of these difficulties. Some people with TSC have very few, while others will have many of them.

Each person with TSC will therefore have their own TAND profile, and this profile may change over time. This checklist was developed to help clinical teams, individuals with TSC and their families
a) *screen for TAND at every clinic visit* and b) *prioritize what to do next*.

Instructions for use

The TAND Checklist was designed to be completed by a clinician with relevant knowledge and experience in TSC, *in partnership* with individuals with TSC or their parents/carers.

The Checklist should take about **10 minutes** to complete.

Where individuals answer **YES** to an item, the clinician should explore the difficulty in sufficient detail to help guide decisions about further evaluation or treatment. **All items should be completed.**

About the interview

Name of TSC Subject: DOB: / / Age:

Name of Interviewer: Date of interview: / /

Name of interviewee: **Self** (circle)

Let's begin

As you will know, the majority of people with TSC have some difficulty in learning, behaviour, mental health, specific aspects of their development and so on. We are going to use this checklist to help us check for these kinds of difficulties. I am going to ask you a number of questions.

Some may be directly relevant; some might not be relevant at all. Just answer as best as you can. At the end I will check to see if there are any additional difficulties we didn't talk about.

For parents/carers of individuals with TSC, please start with question 1.

For individuals with TSC who complete this about themselves, please start with question 3.

01 Let's begin by talking about [subject]'s development to get a sense of where they are at. How old was [subject] when he/she:

- | | | |
|---|---------------------------|-----------------------------------|
| a. First smiled? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| b. Sat without support? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| c. Walked without holding on? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| d. Used single words other than "mama" or "dada"? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| e. Used two words/short phrases? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| f. Was toilet trained during the day? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |
| g. Was toilet trained at night? | Age: <input type="text"/> | Not yet: <input type="checkbox"/> |

02

What is [subject]'s current level of (please tick):

- a. Language: non-verbal simple language fluent
b. Self-care: dependent on others some self-care skills independent
c. Mobility: wheelchair needs significant support some difficulty completely mobile

03

Let's talk about behaviours causing concern to you or to other people.
Have/has [subject] ever had difficulty with any of the following?

- a. Anxiety NO YES
b. Depressed mood NO YES
c. Extreme shyness NO YES
d. Mood swings NO YES
e. Aggressive outbursts NO YES
f. Temper Tantrums NO YES
g. Self-injury, such as hitting self, biting self, scratching self NO YES
h. Absent or delayed onset of language to communicate NO YES
i. Repeating words or phrases over and over again NO YES
j. Poor eye contact NO YES
k. Difficulties getting on with other people of similar age NO YES
l. Repetitive behaviours, such as doing the same thing over and over again NO YES
m. Very rigid or inflexible about how to do things or not liking change in routines NO YES
n. Overactivity/hyperactivity, such as being constantly on the go NO YES
o. Difficulty paying attention or concentrating NO YES
p. Restlessness or fidgetiness, such as wriggling or squirming NO YES
q. Impulsivity, such as butting in, not waiting turn NO YES
r. Difficulties with eating, such as eating too much, too little, unusual things NO YES
s. Sleep difficulties, such as with falling asleep or waking NO YES
If you answered YES to any of the above:
Have you had further evaluation or support for it? NO YES
Would you like to have further evaluation or support for it? NO YES

04

Problem behaviours may add up to meet criteria for specific psychiatric disorders. Have/has [subject] ever received a diagnosis of:

- a. Autism Spectrum Disorder (ASD), including autism, Asperger's NO YES
b. Attention Deficit Hyperactivity Disorder (ADHD) NO YES
c. Anxiety Disorder, including as panic, phobia, separation anxiety disorder NO YES
d. Depressive Disorder NO YES
e. Obsessive Compulsive Disorder NO YES
f. Psychotic Disorder, including schizophrenia NO YES
If you answered YES to any of the above
Have you had further evaluation or support for it? NO YES
Would you like to have further evaluation or support for it? NO YES

05 About half of people with TSC will have significant difficulties in their overall intellectual development and may have 'intellectual disability'.

- a. Have you ever been concerned about this for [subject]? NO YES
- b. Have/has [subject] ever had a formal evaluation of intelligence by a professional using IQ-type tests? NO YES
 If YES, what did results show? Normal Intellectual Ability (IQ > 80)
 Borderline Intellectual Ability (IQ 70-80)
 Mild Intellectual Disability (IQ 50-69)
 Moderate Intellectual Disability (IQ 35-49)
 Severe Intellectual Disability (IQ 21-34)
 Profound Intellectual Disability (IQ <20)
- c. What is your view of [subject]'s intellectual ability? Normal Intellectual Ability
 Mild-Moderate Intellectual Disability
 Severe - Profound Intellectual Disability
- d. Would you like to have further evaluation or support for it? NO YES

06 Many people with TSC who are of school age will have difficulty in school. [For individuals of school age]: Does/do [subject] have any difficulty with any of the following: [For individuals after school age]: Did [subject] have any difficulty with any of the following:

- a. Reading N/A NO YES
- b. Writing N/A NO YES
- c. Spelling N/A NO YES
- d. Mathematics N/A NO YES
- If you answered YES to any of the above**
- Have/has [subject] had further evaluation or support for it? NO YES
- Have/has [subject] been considered for any additional support in school such as extra help or an Individual Educational Plan (IEP)? NO YES
- Would you like to have further evaluation or support for [subject]? NO YES

07 The majority of people with TSC will have some difficulties in some specific brain skills. Do/does [subject] have difficulty with any of the following:

- a. Memory, such as remembering things that have happened NO YES
- b. Attention, such as concentrating well, not getting distracted NO YES
- c. Dual-tasking/ Multi-tasking, such as doing 2 tasks at the same time NO YES
- d. Visuo-spatial tasks, such as solving puzzles or using building blocks NO YES
- e. Executive skills, such as planning, organizing, flexible thinking NO YES
- f. Getting disoriented, such as not knowing the date or where you are NO YES
- If you answered YES to any of the above**
- Have/has [subject] had further evaluation or support for it? NO YES
- Would you like to have further evaluation or support for these difficulties? NO YES

08 Apart from the challenges listed above, TSC can have a big impact on people's lives in other ways. Have/has [subject] had any difficulties with:

- a. Low self-esteem NO YES
- b. Very high levels of stress in families, for instance between *siblings* NO YES
- c. Very high levels of stress between *parents* leading to significant relationship difficulties NO YES

If you answered YES to any of the above
 Have/has [subject] and/or your family had further evaluation or support for it? NO YES
 Would you like to have further evaluation or support for it? NO YES

09 Taking together all the difficulties discussed above, how much have these bothered, troubled or distressed you/your child/family?



10 Of all the concerns listed above, what are your top priorities to work on next?

- a.
- b.
- c.

11 Do you have any other worries about TAND for [subject] that we have not talked about as we went through the checklist?

NO YES If YES, please list:

.....

.....

.....

.....

Thank You!

12 Interviewer's judgement of impact/burden on the individual/child/family.



The TAND Checklist is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licence (<http://creativecommons.org/>). The TAND Checklist may be copied, reproduced and used in paper format for non-commercial purposes provided that the original work is properly credited and that no changes are made to the checklist. For any enquiries regarding use of the TAND Checklist, please contact Prof Petrus de Vries (petrus.devries@uct.ac.za).

Appendix B: HREC Approval



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 September 2015

HREC REF: 340/2015

Prof P de Vries
Child & Adolescent Psychiatry
Red Cross War Memorial Children's Hospital

Dear Prof de Vries

PROJECT TITLE: TSC-ASSOCIATED, NEUROPSYCHIATRIC DISORDERS (TAND): CLUSTER IDENTIFICATION, TOP-TEN PRIORITISATION, AND TOOLKIT DEVELOPMENT (PhD-candidate-L Leclizio)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 10 September 2015.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the PhD student, Loren Leclizio will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

HREC 340/2015

Appendix C: Informed Consent

UNIVERSITY OF CAPE TOWN DIVISION OF CHILD AND ADOLESCENT PSYCHIATRY



PATIENT INFORMATION SHEET

Principal Investigator: Professor Petrus de Vries

Ph.D student and project lead: Loren Leclezio

This study is for degree purposes

TSC-Associated Neuropsychiatric Disorders (TAND) cluster investigation, TOP-TEN prioritisation, and toolkit development

Background

A study done in the United Kingdom showed that many people with Tuberous Sclerosis Complex (TSC) do not get tested for neuropsychiatric disorders such as mood disorders, anxiety, autism spectrum disorders, attention difficulties and many more. As a result many children and adults with TSC do not get the necessary treatment. In 2012 an international panel of experts in TSC decided to develop a checklist to help doctors, nurses and other health care professionals to identify these difficulties. Many professionals and families feel overwhelmed by the complexity of these disorders and say that they do not know where to start and how to access relevant information, tips or 'next step' approaches. In this project we want to see if there are natural TAND Clusters/Groups – combinations of behaviours across different levels - that will simplify further evaluations and management.

What will I have to do?

We would like to invite you to join us for a focus group discussion about TSC. In

the focus group there will be 8-15 people. We will discuss various treatment options, resources, websites, tips and tools that you have found useful. These questions will relate to the following TSC-Associated Neuropsychiatric Disorders (TAND):

1. Behavioural items
2. Psychiatric items
3. Intellectual disability
4. Difficulties at school
5. Difficulties with specific brain skills

You will have the opportunity to write down 1-3 helpful resources etc. for each question, your responses will be kept anonymous. Thereafter, the group will discuss the responses provided to help us identify the top resources, tips and tools to be included in a draft TAND toolkit.

How long will all of this take?

The focus group should not last more than about 2 hours.

What will you do with the information I give you?

Your feedback will be used to generate a draft TAND toolkit.

What will happen to the results/data?

We will analyse all the data, and use your feedback in the development of a draft TAND Toolkit. We will prepare academic manuscripts and send it to scientific journals for publication and will present it at conferences to tell other people about what we have done.

Will I get paid for taking part?

You will not get paid for participating in the focus group, but all your reasonable travel expenses to participate will be refunded. We will give you a small amount of money as a thank you for helping us. We will also give you refreshments during the focus group.

How will this affect me or my child's treatment?

Whether you decide to take part or decide not to, it will have no affect on the standard of care that you or your child is currently receiving. You may withdraw

from the study at any point.

Will the discussions be confidential?

Participants in the focus group will be reminded to respect confidentiality of others and not talk about what they said outside the group. The information given by people in the focus group will not be linked to their names in any way. The recording for the focus group will be destroyed after it has been transcribed and typed up by the research team.

If you have any questions or queries about the research or about helping us in our research, please contact Loren Leclizio: (cell) 082 4420351 (email)

*loren.leclizio@uct.ac.za or Professor de Vries, [\(telephone\) +2721 6854103](tel:+27216854103)
[\(email\) petrus.devries@uct.ac.za](mailto:petrus.devries@uct.ac.za)*

You can also contact your local TSC physician or organization for more information, herewith contact details:

.....

This is phase II(b) of a larger study looking at identifying natural clusters/groupings of TSC-Associated Neuropsychiatric Disorders (TAND). In this phase we hope to obtain your feedback on resources, references, tips and tools that you have found useful and would recommend other families and professionals consider. We would also like your recommendations in terms of the best format in which to make this information available. This information will be used to generate a draft TAND toolkit.

Risks & Benefits

This study involves no more than minimal risk. The information gathered from the research team will be available to the treating physicians and clinical team working with the TSC patients. Findings from the study will be used in the development of a TAND toolkit to assist clinicians and families/individuals with TSC in to identify TAND early, to effectively manage clinical, psychological and social needs, and lastly to inform treatment options.

End of Study

At the end of the study (which involves two phases), two to three manuscripts will be prepared for submission to peer-reviewed international journals. The results will be submitted for presentation at international conferences relevant to TSC and the findings will be shared with participating organisations and individuals in appropriate formats.

The study has been explained to me, and my questions have been answered. I understand that participation in this study is voluntary, and that I may withdraw from the study at any point. I understand that confidentiality cannot be assured when participating in a focus group. Outcomes from the focus group will be anonymous. I consent to participate in this study.

Do you agree to have the discussions recorded for later analysis? **Yes No**

You will not be able to partake in this phase of the research if you do not wish to be audio-recorded.

Name:.....

Signature:.....

Date:

I have explained the study to the participant, and in my opinion s/he understands that participation is voluntary and is able to give informed consent.

Researcher:.....**Signature:**.....

Date:

Loren Leclezio

Ph.D student

Division of Child and Adolescent Psychiatry

University of Cape Town

Loren.leclezio@uct.ac.za

(082 4420351)

Prof Petrus de Vries

Sue Struegmann Professor of

Child and Adolescent Psychiatry

University of Cape Town

petrus.devries@uct.ac.za

(021) 6854103

Participants in this study may contact the **UCT Faculty of Health Sciences Human Research Ethics Committee (HREC)** with any ethical concerns or questions about your welfare as a study participant.

Room E52-24 Old Main Building

Groote Schuur Hospital

Observatory 7925

sumayah.ariefdien@uct.ac.za (+2721 4066338)

Appendix D: Chapter 4 supporting material

Additional File 1: R code for analysis of data

```
# code Yes = 1, No = 0
dat.coded <- matrix(0, nrow=nrow(datmat), ncol=ncol(datmat))
for (j in 1:ncol(datmat))
  dat.coded[,j] <- as.numeric(datmat[,j])-1
# compute mean square contingency coefficient from coded data
cormat <- cor(dat.coded, use="pairwise.complete.obs")
# convert correlation to dissimilarity
D.mat <- 1-cormat
dimnames(D.mat) <- dimnames(cormat) <-
list(colnames(datmat),colnames(datmat))
plot(hclust(d=as.dist(D.mat), method="ward.D2"))
plot(hclust(d=as.dist(D.mat), method="complete"))
plot(hclust(d=as.dist(D.mat), method="average"))
plot(hclust(d=as.dist(D.mat), method="mcquitty"))
require(cluster)
ave.sil.wd <- rep(NA, 10)
for (k in 2:10)
  ave.sil.wd[k] <- pam(as.dist(D.mat), k=k)$silinfo$avg.width
plot(1:10, ave.sil.wd, type="b")
# optimal number of clusters = max ave sil width
out <- pam(as.dist(D.mat), k=6)$clustering
out <- fanny(as.dist(D.mat), k=6)$clustering
plot(diana(as.dist(D.mat)))
# chosen cluster solution
clus.out <- cutree(hclust(d=as.dist(D.mat), method="ward.D2"),k=6)
elements <- levels(factor(clus.out))
G <- matrix(0, nrow = length(clus.out), ncol = length(elements))
colnames(G) <- elements
for (i in 1:length(elements)) G[clus.out == elements[i], i] <- 1
# Factor analysis
require(psych)
# Congruence
congru <- function(datmat, nfactors=6, fm="minres", rotate="oblimin")
{
  fa.solution <- fa(datmat, nfactors=nfactors, fm=fm, rotate=rotate)
```

```

PHI <- factor.congruence(fa.solution$loadings, G)
I <- diag(max(nrow(PHI),ncol(PHI)))
while (nrow(PHI)<nrow(I)) PHI <- rbind(PHI, 0)
while (ncol(PHI)<ncol(I)) PHI <- cbind(PHI, 0)
SVD <- svd(PHI)
Q <- SVD$v %*% t(SVD$u)
nrow(PHI) - sum(diag(PHI%*%Q))
}
# different FA combinations
combinations <- expand.grid (nfactors = 4:7, fm = c("minres",
"wls", "gls", "pa", "minchi"), rotate = c("varimax",
"quartimax", "bentlerT", "equamax", "varimin", "geominT",
"bifactor", "promax", "oblimin", "simplimax", "bentlerQ",
"geominQ", "biquartimin", "cluster"))
out <- rep(NA,nrow(combinations))
for (i in 1:nrow(combinations))
out[i] <- congru (dat.coded, nfactors=combinations[i,1], fm =
levels(combinations[i,2])[combinations[i,2]], rotate =
levels(combinations[i,3])[combinations[i,3]])
# Estimating factor scores
FA.scores <- function(datmat, nfactors=6, fm="minres",
rotate="bentlerT", scores="regression", cut=0.4)
{
fa.solution <- fa(datmat, nfactors=nfactors, fm=fm, rotate=rotate,
scores=scores)
load.mat <- fa.solution$loadings
score.mat <- fa.solution$scores
FA.mat <- factor2cluster(fa.solution$loadings, cut=cut)
datmat <- na.omit(datmat)
missing.rows <- attr(datmat,"na.action")
cor.vec <- rep(NA, nfactors)
if (ncol(FA.mat)==nfactors)
for (k in 1:nfactors)
{
F.vars <- datmat[,FA.mat[,k]!=0,drop=F]
L.weights <- load.mat[FA.mat[,k]!=0,k]
if (ncol(F.vars)>0)
{
F.obs <- apply(F.vars, 1, sum)/ncol(F.vars)
F.weighted <- rep(NA,length(F.obs))
for (h in 1:length(F.obs))
F.weighted[h] <-

```

```
sum(F.vars[h,]*L.weights)/sum(L.weights)
F.scores <- score.mat[-missing.rows,k]
F.vars <- F.vars[order(F.scores),]
F.obs <- F.obs[order(F.scores)]
F.weighted <- F.weighted[order(F.scores)]
F.scores <- F.scores[order(F.scores)]
x.vals <- barplot (F.scores, las=2)
lines (x.vals, F.obs, col="blue", lwd=2)
title (main=paste(colnames(F.vars),collapse="/"))
}
}
}
FA.scores (dat.coded)
```

Additional File 2: Computation of single congruence index value

Let t_{ij} indicate the Tucker index between factor i and cluster j . If there is m factors and n clusters, the matrix of Tucker index values is $T: m \times n$. If the agreement is perfect, each diagonal value will be 1 and each off diagonal value will be 0. The single congruence value is therefore chosen to measure how close T is to the indicator matrix.

Orthogonal Procrustes Analysis (Gower, 1971) is a method to fit one matrix onto another through multiplication by an orthogonal matrix, which is essentially a high dimensional rotation of T to optimally match A by minimising

$$\|T - AT^{-1}A'\|_F = \text{trace}((T - AT^{-1}A')(T - AT^{-1}A)')$$

The solution is given by $T = AA'$ where the singular value decomposition of $A'A = UDU'$.

To optimally fit T to $A: m \times n$ the two matrices needs to be of commensurable size. This is the case when $m = n$. For $m \neq n$, padding is used where if $m <$

n , $A: m \times n$ is replaced by $A*: m \times n = \begin{pmatrix} A \\ 0 \end{pmatrix}$

while if $m > n$, T is replaced by

$$T*: m \times n = \begin{pmatrix} T & 0 \end{pmatrix}.$$

The difference between the perfect fit measure of one for each factor/cluster pair and the optimally fitted T is measured by the difference in the trace of the two matrices

$$\max \|T, T - AA'(AA')$$

This measure focus on the matching diagonal elements, rather than the overall fit of the optimisation criterion. Perfect fit will yield a value of zero, and the larger the measure, the less congruence.

Appendix E: Chapter 5 supporting material

```

# Sugnet Lubbe, Department of Statistics and ActSci, March 2017
# -----

full.data <- read.csv ("TAND cluster Master dataset Phase 1b.csv")

dat.coded <- matrix(0, nrow=nrow(full.data), ncol=ncol(full.data)-5,
                  dimnames=list(full.data[,1],
                                c(colnames(full.data) [c(3:27,31:43)], "ID")))
temp.dat <- full.data[,c(3:27,31:43)]
for (j in 1:ncol(temp.dat))
  dat.coded[,j] <- as.numeric(temp.dat[,j])-1
dat.coded[,ncol(dat.coded)] <- full.data$IMP.0.10

head(dat.coded)

clustering.data <- dat.coded[,c(1:19,26:35)]
head(clustering.data)

# --- correlation matrix

cormat <- cor(clustering.data, use="pairwise.complete.obs")
dim(cormat)
D.mat <- 1-cormat
dimnames(D.mat) <- dimnames(cormat) <-
  list(colnames(clustering.data), colnames(clustering.data))

plot(hclust(d=as.dist(D.mat), method="ward.D2"))
rect.hclust (hclust(d=as.dist(D.mat), method="ward.D2"), k=7)
Ward.cluster7 <- cutree(hclust(d=as.dist(D.mat), method="ward.D2"),
                       k=7)
Ward.cluster7

#
=====
=====
# Factor analysis

require(psych)

# Congruence
congru <- function(datmat, nfactors=6, fm="minres", rotate="oblimin",
                  G=G7)
{
  fa.solution <- fa(datmat, nfactors=nfactors, fm=fm, rotate=rotate)
  PHI <- factor.congruence(fa.solution$loadings, G)
  I <- diag(max(nrow(PHI), ncol(PHI)))
  while (nrow(PHI)<nrow(I)) PHI <- rbind(PHI, 0)
  while (ncol(PHI)<ncol(I)) PHI <- cbind(PHI, 0)

  SVD <- svd(PHI)
  Q <- SVD$v %*% t(SVD$u)
  nrow(PHI) - sum(diag(PHI%*%Q))
}

#--- 7 clusters Ward
clus.out <- Ward.cluster7
elements <- levels(factor(clus.out))
G7 <- matrix(0, nrow = length(clus.out), ncol = length(elements))
colnames(G7) <- elements

```

```

for (i in 1:length(elements)) G7[clus.out == elements[i], i] <- 1

# different FA combinations
combinations <- expand.grid (nfactors = 4:7, fm = c("minres", "wls",
  "gls", "pa", "minchi"), rotate = c("varimax", "quartimax",
  "bentlerT", "equamax", "varimin", "geominT", "bifactor", "promax",
  "oblimin", "simplimax", "bentlerQ", "geominQ", "biquartimin",
  "cluster"))
combinations <- combinations[-c(264,280),]
out <- rep(NA,nrow(combinations))
for (i in 1:nrow(combinations))
  { print(combinations[i,])
    out[i] <- congru (clustering.data, nfactors=combinations[i,1], fm =
      levels(combinations[i,2])[combinations[i,2]], rotate =
      levels(combinations[i,3])[combinations[i,3]], G=G7)
  }

out<-data.frame(combinations,out)[order(out),]
out

fa(clustering.data, nfactors=7, fm='pa', rotate='cluster')

# --- Bootstrap analysis

plot(hclust(d=as.dist(D.mat), method="ward.D2"))
rect.hclust (hclust(d=as.dist(D.mat), method="ward.D2"), k=7)
Ward.cluster <- cutree(hclust(d=as.dist(D.mat), method="ward.D2"),
  k=7)
Ward.cluster

bootrep <- function(datmat, k=6)
{
  cormat <- cor(datmat, use="pairwise.complete.obs")
  D.mat <- 1-cormat
  dimnames(D.mat) <- dimnames(cormat) <-
    list(colnames(datmat), colnames(datmat))
  cutree(hclust(d=as.dist(D.mat), method="ward.D2"), k=k)
}

bootrep(clustering.data[sample(1:nrow(clustering.data),20),])

require(cluster)
n <- nrow(clustering.data)
B <- 1000
boot.samples <- matrix(sample(1:n, size=n*B, replace=T), ncol=B)
boot.out <- apply(boot.samples,2,function(x)
  bootrep(clustering.data[x,]))

# --- Reliability measures for clusters and factors

Chronbach <- function (X)
{
  k <- ncol(X)
  var.vec <- apply(X, 2, var, na.rm=T)
  sum.Q <- apply(X, 1, sum, na.rm=T)
  var.sum <- var(sum.Q)
  return ((k/(k-1))*((var.sum-sum(var.vec))/var.sum))
}

alpha.if.deleted <- function(X)

```

```

{
  out <- rep(NA, ncol(X))
  for (i in 1:ncol(X))
    out[i] <- Chronbach(X[,-i,drop=F])
  if (!is.null(colnames(X))) names(out) <- colnames(X)
  return(out)
}

head(clustering.data)

# --- Clusters
for (i in 1:7)
  {
    X <- clustering.data[,Ward.cluster7==i]
    print(paste(names(Ward.cluster7)[Ward.cluster7==i],sep=","))
    print (Chronbach(X))
    print (alpha.if.deleted(X))

    cat("=====\n")
  }

# --- Factors
load.mat <- fa(clustering.data, nfactors=7, fm='pa',
  rotate='cluster')$loadings
FA.mat <- apply (load.mat, 2, function (x, cut) as.numeric(x>=cut),
  cut=0.35)
for (i in 1:ncol(FA.mat))
  {
    X <- clustering.data[,FA.mat[,i]==1]
    print(paste(names(Ward.cluster7)[FA.mat[,i]==1],sep=","))
    print (Chronbach(X))
    print (alpha.if.deleted(X))

    cat("=====\n")
  }

head (clustering.data)
ASDclus <- c(13,9,12,10,11,7,8)
ASDclus <- clustering.data[,ASDclus]
head (ASDclus)

Chronbach(ASDclus)
alpha.if.deleted(ASDclus)

```