



# Generalized anxiety disorder in the elderly : role of bio-environmental factors and genetic vulnerability

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# THÈSE

Pour obtenir le grade de  
Docteur

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Biologiques pour la Santé**

**Et de l'unité de recherche Inserm U1061**

**Spécialité : Biologie Santé**

**Présentée par Xiaobin Zhang**

**Generalized anxiety disorder in the elderly: role of  
bio-environmental factors and genetic vulnerability**

Soutenue le 23 Septembre 2016 devant le jury composé de

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## LIST OF ABBREVIATIONS

5-HTT	5-hydroxytryptamine (serotonin) transporter
5-HTTLPR	Serotonin transporter long promoter region
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit hyperactivity disorder
ADR	Adrenergic receptor (-A1A: alpha-1A, -A2A: alpha-2A, -B2: beta2)
AMSTEL	Amsterdam Study of the Elderly
ANSMHWP	Australian National Survey of Mental Health and Well-Being
AUDADIS	The Alcohol Use Disorders and Associated Disabilities Interview Schedule
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CBT	Cognitive Behavioral Therapy
CGI	Clinical global impression (-I: of improvement; -S: severity)
CI	Confidence interval
CIDI	Composite International Diagnostic Interview
COMT	Catechol-O-methyl-methyltransferase
CRH	Corticotropin-releasing hormone (R1: receptor 1)
DAT1	Dopamine active transporter 1
DIS	Diagnostic Interview Schedule
DRD	Dopamine receptor D
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECA	Epidemiologic Catchment Area Program
ESEMeD	European Study of the Epidemiology of Mental Disorders
ESPRIT	Enquête de Santé Psychologique-Risques, Incidence et Traitement
ESR	Estrogen receptor
EWAS	Epigenome-wide association Study
FKBP5	FK506 binding protein 5
GABA	Gamma-aminobutyric acid
GAD	Generalized anxiety disorder
GADIS	Generalized Anxiety Disorder Impact Survey
GHQ	General Health Questionnaire
GMS-AGECAT	Geriatric Mental State Automated Geriatric Examination for Computer Assisted Taxonomy

GWAS	Genome-wide association study
HADS-A	Hospital Anxiety and Depression Scale-Anxiety
HAMA	Hamilton Anxiety Rating Scale
HPA	Hypothalamic-Pituitary-Adrenal
HR	Hazard ratio
ICD	International Classification of Diseases
IQR	Interquartile range
LASA	Longitudinal Aging Study Amsterdam
MAO	Monoamine Oxidases
MHGP	Mental Health in General Population
MINI	Mini-International Neuropsychiatric Interview
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
NCS	National Comorbidity Survey (A: Adolescent Supplement, R: Replication)
NESARC	National Epidemiologic Survey on Alcohol and Related Conditions
NPY	Neuropeptide Y
NR3C (1 or 2)	Nuclear Receptor Subfamily 3, Group C (Member 1 or 2)
NSMHW	National Survey of Mental Health and Wellbeing
OCD	Obsessive-compulsive disorder
OR	Odd ratio
PACAP	Pituitary adenylate cyclase-activating peptide
PCC	Posterior cingulate cortex
PCR	Polymerase chain reaction
PFC	Prefrontal cortex
PTSD	Posttraumatic stress disorder
RCT	Randomized Clinical Trial
RGS	Regulator of G-protein signaling
SCID	Structured Clinical Interview for DSM Disorders
SNP	Single Nucleotide Polymorphism
SNRI	Serotonin-noradrenaline reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TCF7L2	Transcription factor 7-like 2
TMT	Trail Making Test
TPH	Tryptophan hydroxylase
VNTR	Variable number of tandem repeat (u: upstream)

WHR	Waist-to-hip ratio
WMH	World Mental Health Survey

## RESUME

Le trouble anxieux généralisé (TAG) est un problème majeur de santé publique. Il est fréquemment sous ou mal diagnostiqué, notamment chez les personnes âgées. Il est souvent comorbide et source d'incapacité et présente un faible taux de rémission complète. Malgré des conséquences socio-économiques et sanitaires importantes, les recherches sur les déterminants du TAG en population générale âgée restent limitées. L'objectif de cette thèse était d'étudier les caractéristiques cliniques et étiologiques du TAG à partir de l'étude prospective ESPRIT, constituée de 2259 personnes âgées de 65 ans et plus et examinées à 6 reprises pendant 12 ans.

La prévalence du TAG des 6 derniers mois était de 4,6%, 14% des cas présentant une comorbidité avec une dépression majeure et 35% avec une phobie mais les facteurs associés sont différents. Au cours des 12 ans de suivi, 8,4% des participants ont présenté un TAG incident (10 pour 1000 personnes-années), qui était un premier épisode dans 80% des cas. Plusieurs facteurs prédictifs de TAG ont été identifiés à partir de modèles statistiques multivariés : le sexe féminin, la survenue d'événements de vie stressants récents ou anciens (pendant l'enfance), certaines maladies chroniques (troubles respiratoires, cognitifs, arythmie et insuffisance cardiaque, dyslipidémie, adiposité) et troubles psychiatriques (dépression majeure, phobie, antécédents de TAG). Certains variants génétiques des récepteurs adrénérgiques augmentent le risque de TAG (mais pas de phobie) et peuvent moduler l'effet des événements stressants. Le TAG apparaît comme un trouble affectif multifactoriel lié au stress résultant de facteurs de risque proximaux et distaux et cliniquement distinct des autres troubles psychiatriques majeurs de la personne âgée.

Ce travail apporte de nouvelles connaissances sur les déterminants du TAG avec des implications cliniques en termes de diagnostic et d'étiologie. Il pourrait permettre le développement de stratégies originales de prévention et d'intervention chez le sujet âgé, avec des conséquences majeures en santé publique.

Mots clés : Psychiatrie, troubles anxieux, stress, récepteurs adrénérgiques, épidémiologie, cohorte, facteurs de risque, personne âgée.

## ABSTRACT

Generalized anxiety disorder (GAD) is a major public health concern. It is frequently under-recognized or misdiagnosed, especially in the elderly. It is associated with high comorbidity and disability, and a low rate of full remission. Despite substantial socioeconomic and public health consequences, there has been little research to identify GAD determinants in general elderly populations. The aim of this thesis was to provide a deeper understanding of the clinical characteristics and risk factors for GAD in late life from the prospective ESPRIT study of 2259 community-dwelling French elderly (aged 65 years and over) examined six times over 12 years.

The 6-month current prevalence of GAD was 4.6%, 14% of the cases were comorbid with major depression and 35% with phobia but the factors associated with these disorders differed. During the 12-year follow-up, 8.4% of the participants experienced incident GAD (10 per 1000 person-years) of which 80% were first episodes. Multivariate statistical models showed that the main predictors for late-life GAD were being female, reporting recent and childhood adverse life events, having chronic physical (respiratory disorders, arrhythmia and heart failure, cognitive impairment, dyslipidemia, and adiposity), and mental (major depression, phobia, and past GAD) disorders. Specific genetic variants of adrenergic receptors increase the risk of GAD (but not phobia) and moderate the effect of adverse life events. GAD can be characterized as a multifactorial stress-related affective disorder resulting from both proximal and distal risk factors, and is clinically distinct from other major psychiatric disorders in the elderly.

This work provides new knowledge on GAD determinants with clinical implications for diagnosis and etiology. It could also contribute to developing novel preventative and intervention strategies in the elderly, with major potential consequences for overall health and daily functioning.

Key words: Psychiatry, anxiety disorder, stress, adrenergic receptors, epidemiology, cohort, predictors, elderly.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	3
LIST OF ABBREVIATIONS .....	4
RESUME .....	7
ABSTRACT .....	8
SYNOPSIS EN FRANÇAIS .....	11
SECTION 1 – INTRODUCTION .....	18
CHAPTER 1 – Epidemiology of anxiety disorders.....	19
1.1 Definitions and lifetime prevalence of anxiety disorders .....	19
1.2 Age of onset and course of anxiety disorders.....	25
1.3 Current prevalence rates of anxiety disorders in the elderly .....	25
1.4 Comorbidity .....	26
1.5 Disability and mortality risk associated with anxiety disorders .....	27
1.6 The economic burden of anxiety disorders.....	27
CHAPTER 2 - Generalized anxiety disorder (GAD): general characteristics .....	29
2.1 Definitions and diagnosis criteria .....	29
2.2 GAD prevalence .....	30
2.3 Age of onset and course.....	31
2.4 Psychiatric comorbidity .....	31
CHAPTER 3 - Treatments of GAD.....	33
3.1 Psychotherapy .....	33
3.2 Pharmacotherapy .....	34
CHAPTER 4 - Neurobiology of GAD.....	37
4.1 Neuroimaging structural and functional modifications .....	37
4.2 Neuroendocrine and neurotransmitter systems.....	38
CHAPTER 5 - Risk factors for GAD .....	42
5.1 Socio-demographic factors associated with GAD.....	42
5.2 Lifestyle characteristics .....	43
5.3 Physical health .....	43
5.4 Stressful life events, parental characteristics, and history of psychiatric disorder .....	44
CHAPTER 6 - Heritability and genetic risk factors for GAD.....	50
6.1 Heritability estimates: family and twin studies .....	50
6.2 Candidate-gene studies of GAD .....	50
6.3 Genome-wide association studies (GWAS) of GAD .....	55
SECTION 2 - THESIS HYPOTHESES AND OBJECTIVES.....	60
SECTION 3- SUBJECTS & METHODS .....	62

CHAPTER 1 - The Esprit study .....	63
1.1 Study design and population.....	63
1.2 Measures .....	65
CHAPTER 2 - Statistical analyses .....	70
2.1 Statistical methods .....	70
2.2 Statistical Models.....	72
SECTION 4 - RESULTS.....	74
CHAPTER 1 - Study design and sample.....	75
1.1 Description of the overall design and general flowchart .....	75
1.2 Description of the Esprit cohort at baseline.....	77
1.3. Rationale and objectives of the work.....	80
CHAPTER 2 - Articles .....	82
2.1 Generalized anxiety in community-dwelling elderly: prevalence and clinical characteristics	82
2.2 Risk factors for late-onset generalized anxiety disorder: results from a 12-year prospective cohort (The Esprit study) .....	83
2.3 Further evidence for a role of the adrenergic nervous system in generalized anxiety disorder	84
SECTION 5 – DISCUSSION.....	85
CHAPTER 1 - Prevalence and incidence rates of late-life GAD .....	86
CHAPTER 2 – Current psychiatric comorbidity with GAD .....	87
CHAPTER 3 – Use of psychotropic medications.....	88
CHAPTER 4 – Factors associated with late-life GAD.....	89
4.1 General characteristics of late-life GAD .....	89
4.2 Socio-demographic and lifestyle characteristics .....	91
4.3 Stressful life events.....	91
4.4 Physical health .....	92
4.5 Mental health .....	94
CHAPTER 5 – Genetic vulnerability related to adrenergic receptors.....	96
CHAPTER 6 – Limitations and Strengths.....	98
6.1 Limitations .....	98
6.2 Strengths .....	98
SECTION 6 – CONCLUSION AND PERSPECTIVES.....	100
CHAPTER 1– Main conclusions.....	101
CHAPTER 2– Perspectives .....	102
SECTION 7 - REFERENCES.....	103
INDEX OF TABLES.....	<b>Error! Bookmark not defined.</b>
INDEX OF FIGURES .....	143

## **SYNOPSIS EN FRANÇAIS**

### **Anxiété généralisée du sujet âgé : rôle des facteurs bio-environnementaux et de la vulnérabilité génétique**

#### **SECTION 1 : INTRODUCTION**

##### **Chapitre 1 : Epidémiologie des troubles anxieux**

L'allongement de la durée de vie s'accompagne d'une augmentation de la prévalence des maladies liées au vieillissement, notamment de certaines pathologies mentales comme les troubles anxieux. Actuellement, on estime que 10% à 20% des personnes dans le monde présentent un trouble anxieux soit en Europe près de 61,5 millions de personnes (Wittchen et al., 2011). Ces pathologies s'accompagnent d'une forte comorbidité notamment avec la dépression mais aussi d'autres pathologies chroniques (vasculaires...), d'une perte d'autonomie et d'un risque accru de mortalité (Wolitzky-Taylor et al., 2010). Elles constituent donc des enjeux de santé publique majeurs pour lesquels des alternatives thérapeutiques sont activement recherchées.

##### **Chapitre 2 : Caractéristiques générales du trouble anxieux généralisé (TAG)**

Le trouble anxieux généralisé (TAG) est l'un des deux troubles anxieux les plus fréquents avec les phobies. Le TAG est un trouble psychiatrique majeur qui touche près de 10% de la population et présente un faible taux de rémission complète. En Europe, 8,9 millions de personnes souffriraient d'un TAG (Wittchen et al., 2011). Le TAG est souvent comorbide avec d'autres pathologies mentales ou somatiques. Peu de données sont disponibles sur le TAG chez les sujets âgés qui est souvent considéré comme un continuum du trouble observé chez les sujets jeunes ou/et comme un simple marqueur de sévérité d'autres troubles psychiatriques ou somatiques.

##### **Chapitre 3 : Traitement du TAG**

Le TAG est généralement sous ou mal diagnostiqué notamment chez les personnes âgées du fait de la difficulté de reconnaître les caractéristiques cliniques et les manifestations physiques de ce trouble anxieux et d'en identifier les facteurs étiologiques (Hoge et al., 2012). Une conséquence est que le traitement du TAG est souvent limité ou inadapté chez les sujets âgés. Les thérapies les plus fréquemment recommandées sont basées sur les traitements pharmacologiques avec en première intention, les antidépresseurs (inhibiteurs sélectifs de la recapture de sérotonine ou inhibiteurs de la recapture de sérotonine et de la noradrénaline) ou un antiépileptique présentant une activité anxiolytique, la prégabaline et/ou les traitements psychothérapeutiques, en particulier les thérapies comportementales et cognitives (Bandelow et al., 2015b). Les benzodiazépines doivent être utilisées avec précaution chez les sujets âgés et essentiellement pour un traitement adjuvant de courte durée des formes aiguës de l'anxiété. Les traitements doivent être poursuivis plusieurs mois après la rémission des symptômes.

## **Chapitre 4 : Neurobiologie du TAG**

Malgré des conséquences en santé publique et socio-économiques importantes, les recherches sur les mécanismes physiopathologiques impliqués dans l'installation et le maintien du TAG restent limitées. Des modifications cérébrales structurales et fonctionnelles au niveau de l'amygdale et du cortex préfrontal qui sont cruciaux dans la régulation des émotions, ainsi qu'un dysfonctionnement des systèmes neurotransmetteurs (*e.g.* sérotoninergique et GABAergique) et neuroendocriniens (axe hypothalamo-hypophyso-surrénalien, HHS et système adrénergique/ noradrénergique) ont déjà été rapportées dans le TAG, mais les bases neurobiologiques du TAG restent encore peu connues en particulier chez les personnes âgées (Faravelli et al., 2012; Graeff et al., 2010; Hilbert et al., 2014; Martin et al., 2009; Mochcovitch et al., 2014; Ulrich-Lai et al., 2009). Un obstacle majeur, outre la difficulté de reconnaître les caractéristiques cliniques et les manifestations physiques de TAG, reste l'identification des stimuli déclencheurs initiaux et des caractéristiques étiologiques spécifiques du TAG.

## **Chapitre 5 : Facteurs de risque du TAG**

Les études épidémiologiques sur le TAG sont relativement limitées et les causes étiologiques restent en grande partie inconnues. Quelques études ont examiné l'association entre le TAG et des facteurs sociodémographiques, comportementaux, environnementaux ou cliniques mais elles portaient essentiellement sur des enfants ou des jeunes adultes (Moreno-Peral, et al., 2014). En outre, ces études essentiellement transversales n'ont pas permis de différencier les facteurs associés au TAG qui coexistent ou découlent de la psychopathologie, d'avec les facteurs étiologiques de TAG. Les études longitudinales prospectives qui permettent d'éviter ce biais de causalité inverse et de séparer la cause de l'effet sont rares. Deux études prospectives avec un suivi de 3 ans ont été réalisées chez les personnes âgées et seuls le sexe féminin et les antécédents personnels de dépression ou de trouble anxieux ont été identifiés comme facteurs prédictifs de TAG (Chou et al., 2011; Schoevers et al., 2005). L'effet propre des troubles chroniques liés à l'âge (par exemple, les pathologies cardiovasculaires, respiratoires ou métaboliques) n'a pas été évalué prospectivement pas plus que les antécédents familiaux de troubles mentaux ou les environnements défavorables, alors que cette population est plus susceptible d'avoir accumulé les événements stressants au cours de sa vie.

## **Chapitre 6 : Héritabilité et facteurs de risque génétiques du TAG**

Les études les plus récentes ont porté sur la contribution des facteurs génétiques dans l'étiologie du TAG. Les études de familles et de jumeaux ont montré une héritabilité relativement importante des troubles anxieux (30% pour le TAG) et un chevauchement possible avec d'autres troubles psychiatriques (Domschke et al., 2012). Mais jusqu'à présent les études génétiques d'association et les études pangénomiques réalisées sur les troubles anxieux ont le plus souvent été limitées en nombre et en effectifs et n'ont permis l'identification que de quelques variants génétiques. Les rares variants associés au TAG concernaient les systèmes impliqués dans la neurotransmission ou la neurogenèse (transporteur de sérotonine, récepteurs dopaminergiques, monoamine oxydase A, neuropeptide Y, BDNF) mais ces variants ont aussi été associés à d'autres troubles psychiatriques. Dans la majorité des cas il s'agissait d'études cas-témoins, chez des jeunes adultes, n'ayant examiné qu'un seul variant par gène et n'ont pas été répliquées. L'effet modérateur d'interactions plus complexes (gène x environnement) n'a pas été examiné dans le TAG. Des études gènes candidats impliquant une stratégie ciblée sur des mécanismes pathophysiologiques pourraient être plus adaptées pour tester des hypothèses spécifiques concernant l'implication de certains gènes susceptibles de jouer un rôle clé dans l'étiologie de la maladie et les interactions avec des facteurs environnementaux.

## SECTION 2 : HYPOTHESES ET OBJECTIFS DE LA THESE

Au vu de la revue de la littérature, nous avons fait l'hypothèse que le TAG du sujet âgé pourrait avoir des caractéristiques propres, distinctes de celles des sujets jeunes et des autres troubles affectifs. En outre, chez les sujets âgés, l'accumulation de traumatismes au cours de la vie et une mauvaise gestion du stress pourraient conduire à une dysrégulation des systèmes biologiques liés au stress (système nerveux autonome et axe HHS) et un risque accru de TAG chez les sujets présentant une vulnérabilité génétique.

Cette thèse a pour but d'étudier les caractéristiques cliniques et étiologiques du TAG chez les sujets âgés à partir de l'étude prospective ESPRIT, constituée de 2259 personnes âgées examinées à 6 reprises pendant 12 ans. Les 3 objectifs spécifiques sont :

**Objectif 1 :** estimer la prévalence actuelle et vie entière du TAG en population générale âgée, la comorbidité avec d'autres troubles psychiatriques majeurs et décrire certaines de ses caractéristiques sociodémographiques, cliniques et environnementales.

**Objectif 2 :** estimer le taux d'incidence du TAG au cours de 12 ans de suivi et caractériser les facteurs prédictifs du TAG à début tardif parmi une large palette de facteurs sociodémographiques, comportementaux, biologiques, cliniques et environnementaux.

**Objectif 3 :** examiner l'association entre certains variants génétiques impliqués dans le système adrénergique et le TAG du sujet âgé en tenant compte d'interactions (de type gène x environnement) avec la survenue d'événements de vie stressants.

## SECTION 3 : SUJETS ET METHODES

### Chapitre 1 : L'étude Esprit

Les analyses ont été réalisées à partir d'une base de données déjà constituée issue de l'étude longitudinale en population générale, ESPRIT (Enquête de Santé Psychologique – Risques, Incidence et Traitement) (Ritchie et al., 2004), portant sur l'évaluation psychiatrique des personnes âgées. Les principales caractéristiques sont résumées ci-dessous.

Inclusion et suivis. Entre 1999 et 2001, 2259 sujets non-institutionnalisés de 65 ans et plus ont été recrutés par tirage au sort à partir des listes électorales de Montpellier. Les données sociodémographiques, médicales et environnementales ont été recueillies à l'inclusion et un examen clinique approfondi (en particulier neurologique et vasculaire) a été réalisé. Les paramètres biochimiques classiques ont été évalués à partir d'une prise de sang à jeun. Une banque d'ADN a notamment été constituée à partir de prélèvement de cellules buccales. Les sujets ont été suivis 5 fois, 2, 4 et 7, 10 et 12 ans après leur inclusion.

Troubles psychiatriques. Un examen psychiatrique standardisé, le MINI (Mini International Neuropsychiatric Interview) permet d'évaluer la survenue de TAG ainsi que d'autres troubles anxieux (phobie, trouble panique, trouble obsessionnel compulsif et état de stress post-traumatique) et la dépression majeure au cours de la vie (selon les critères du DSM-IV) (Lecrubier et al., 1997)

Evaluation cognitive. Les tests neuropsychologiques utilisés permettent d'évaluer la mémoire visuelle (Test de Benton), la fluence verbale (Isaacs Set Test), les performances visuospatiales et psychomotrices (Trail Making Test A et B) ainsi que le fonctionnement cognitif global (Mini Mental State Examination). Un protocole clinique standardisé a été utilisé pour le diagnostic et la classification des démences à partir des critères du DSM-IV et les cas ont été validés par un groupe indépendant de neurologues.

Événements de vie stressants. Les événements de vie stressants récents ont été évalués à l'aide d'un questionnaire validé, constitué de 12 items évaluant la survenue dans l'année écoulée d'une maladie sévère, du décès d'un proche, de difficultés financières ou judiciaires (Harwood et al., 1998). Les événements de vie survenue ou cours de l'enfance ont été évalués à partir d'un auto-questionnaire qui examine à la fois des facteurs négatifs (*e.g.* abus sévères, séparation ou perte des parents, maladie mentale des parents, conflits, pauvreté...) et des facteurs positifs (enfance heureuse, affection des parents ou d'un adulte...) (Ritchie et al., 2009).

Facteurs de risque génétique. Huit polymorphismes de gènes impliqués dans le système adrénergique (récepteurs adrénergiques alpha(1A), alpha(2A) et beta2 et facteur de transcription TCF7L2) ont été génotypés.

## **Chapitre 2 : Analyses statistiques**

Les analyses statistiques ont été réalisées à partir des données transversales et longitudinales et avec des sujets ne présentant pas de données manquantes sur le TAG et les principales covariables d'intérêt. Les relations avec le TAG prévalent ont été modélisées à l'aide de régressions multivariées, logistiques (lorsque la variable dépendante, TAG, était binaire) ou polytomiques (lorsque la variable TAG était en 3 catégories, dans le cas de l'étude en fonction de la comorbidité avec la phobie). Pour les études génétiques, les interactions gène-environnement avec les événements de vie ont aussi été examinées. Dans les analyses longitudinales, le risque de développer un TAG au cours des 12 ans de suivi a été évalué en utilisant des modèles de survie (modèles de Cox) multivariés chez les participants ne présentant pas de TAG à l'inclusion.

## **SECTION 4 : RESULTATS**

### **Chapitre 1 : Description de l'étude et des échantillons retenus dans chaque analyse**

Ce chapitre présente le schéma des différentes analyses réalisées dans cette thèse, les caractéristiques générales des participants de l'étude ESPRIT à l'inclusion ainsi que le rationnel et les objectifs des études réalisées.

### **Chapitre 2 : Articles**

Ce chapitre présente les principaux résultats obtenus dans cette thèse sous forme de 3 articles, résumés ci-dessous.

#### **Article 1 : TAG en population générale âgée : prévalence et caractéristiques cliniques**

Objectif – Evaluer les taux de prévalence (actuelle et vie entière) du TAG chez des sujets âgés, certaines de ses caractéristiques cliniques (comorbidité, traitements) ainsi que les facteurs de risque associés.

Méthodes – Cette analyse a été réalisée à partir d'un échantillon de 1974 participants de l'étude Esprit en utilisant des modèles de régression logistique multivariés incluant un grand nombre de facteurs sociodémographiques, biologiques et cliniques, le style de vie, et des facteurs environnementaux (événements de vie stressants récents ou anciens).

Résultats – Onze % des participants ont présenté un TAG au cours de leur vie et dans un quart des cas

il s'agissait d'un TAG à début tardif, après 50 ans. La prévalence actuelle du TAG lors des 6 derniers mois était de 4,6 %. La quasi-totalité des cas actuels étaient récurrents mais seulement 36,3% recevaient un traitement antidépresseur ou/et anxiolytique. Quatorze % des participants diagnostiqués avec un TAG présentaient aussi une comorbidité avec une dépression majeure et 35% avec une phobie mais les facteurs associés étaient différents. Le sexe féminin, un faible support affectif pendant l'enfance, une altération de la fluence verbale, une prise plus importante de médicaments somatiques étaient associés à un risque élevé de TAG indépendamment des autres facteurs psychiatriques (prise de médicaments psychotropes, dépression majeure et phobie).

Conclusion – La prévalence du TAG est élevée chez les sujets âgés et on observe un début tardif dans un cas sur 4. Le TAG du sujet âgé n'est pas un simple marqueur de sévérité d'un autre trouble psychiatrique comme la dépression et apparaît cliniquement distinct des autres troubles anxieux majeurs de la personne âgée, comme la phobie.

Production scientifique - Ce travail a fait l'objet d'une publication scientifique dans un journal international :

**Zhang X**, Norton J, Carrière I, Ritchie K, Chaudieu I, Ancelin ML. Generalized anxiety in community-dwelling elderly people: prevalence and clinical characteristics. *Journal of Affective Disorders*, 2014, 172:24-9.

## **Article 2 : Incidence sur 12 ans et facteurs de risque de TAG à début tardif : résultats de l'étude Esprit**

Objectif – Evaluer l'incidence du TAG chez des sujets âgés et identifier les facteurs prédictifs de l'apparition du TAG sur une période de 12 ans.

Méthodes – Le taux d'incidence sur 12 ans a été calculé à partir de 1711 participants sans TAG à l'inclusion. Les facteurs de risque associés à de nouveaux cas de TAG ont été évalués à partir de modèles de Cox multivariés à entrée retardée, incluant un grand nombre de facteurs sociodémographiques, biologiques et cliniques, le style de vie, et des facteurs environnementaux (événements de vie stressants).

Résultats – Au cours des 12 ans de suivi, l'incidence du TAG chez les sujets âgés était de 8,4% (10 pour 1000 personnes-années). Il s'agissait d'un premier épisode (tardif) dans 80% des cas. Plusieurs facteurs prédictifs de TAG ont été identifiés : le sexe féminin, la survenue d'événements de vie stressants récents ou anciens, certaines maladies chroniques (troubles respiratoires, cognitifs, arythmie et insuffisance cardiaque, dyslipidémie) et troubles psychiatriques (dépression majeure, phobie, antécédents de TAG). Parmi les facteurs les plus distaux, des difficultés financières, la séparation ou la perte des parents ou un faible support affectif pendant l'enfance, ainsi que des antécédents de troubles mentaux chez les parents sont aussi des facteurs de risque indépendants de TAG.

Conclusion – Le TAG apparaît comme un trouble affectif multifactoriel lié au stress résultant de facteurs de risque distaux (traumas de l'enfance) et proximaux dont certains sont modifiables et donc potentiellement susceptibles d'interventions ciblées.

Production scientifique - Ce travail a fait l'objet d'une publication scientifique dans un journal international.

**Zhang X**, Norton J, Carrière I, Ritchie K., Chaudieu I, Ancelin ML. Risk factors for late onset generalized anxiety disorder: results from a 12-year prospective cohort (the ESPRIT study). *Translational Psychiatry*, 2015, 5:e536.

### **Article 3 : Implication du système nerveux adrénergique dans le TAG : rôle des récepteurs adrénergiques**

Objectif – Evaluer le rôle de la variabilité génétique au niveau des récepteurs adrénergiques dans la vulnérabilité au TAG chez les sujets âgés.

Méthodes – Cette analyse transversale a été réalisée à partir d'un échantillon de 844 participants de l'étude Esprit ayant bénéficié d'un génotypage. Nous avons examiné la relation entre 8 polymorphismes de gènes impliqués dans le système adrénergique (récepteurs adrénergiques alpha(1A), alpha(2A) et beta2 et facteur de transcription TCF7L2) et le TAG à l'aide d'une régression logistique multivariée ajustée sur l'âge et le sexe et évalué le rôle de médiateurs potentiels (indice de masse corporelle ou dépression majeure). Les interactions avec les événements de vie adverses ont également été examinées.

Résultats – Deux variants du récepteur alpha(1A) et un variant du récepteur beta2 étaient associés à une modification significative de 4 fois du risque de TAG. Par contre, aucune association significative n'a été observée avec la phobie. Certains variants peuvent moduler l'effet des environnements adverses (traumas récents ou faible support affectif pendant l'enfance) sur le risque de TAG (interactions Gène x Environnement).

Conclusion – Certains variants génétiques des récepteurs adrénergiques apparaissent être des facteurs de vulnérabilité du TAG, ce qui confirme le rôle majeur du système nerveux adrénergique et du stress dans la pathogenèse du TAG.

Production scientifique – Ce travail est actuellement soumis pour publication scientifique dans un journal international.

**Zhang X**, Norton J, Carrière I, Ritchie K, Chaudieu I, Ryan J, Ancelin M.L. Further evidence for a role of the adrenergic nervous system in generalized anxiety disorder. Submitted.

## **SECTION 5 : DISCUSSION**

Ces travaux sont discutés au regard de la littérature et fournissent plusieurs informations inédites sur le TAG en population générale âgée. Les taux de prévalence (4,6% en actuel et 11% sur la vie entière) et d'incidence (8,4% sur 12 ans) apparaissent relativement élevés chez ces personnes âgées (**chapitre 1**). Le TAG présente des caractéristiques cliniques propres distinctes d'autres troubles psychiatriques majeurs du sujet âgé et fréquemment comorbides du TAG, comme la dépression ou les phobies (**chapitre 2**). Près de deux tiers des cas de TAG n'ont reçu aucun traitement médicamenteux suggérant une faible fréquence de détection et/ou de traitement de ce trouble chez les personnes âgées en population générale (**chapitre 3**). Les premiers épisodes tardifs sont plus fréquents que décrit précédemment et présentent des facteurs de risque spécifiques, intrinsèques (événements de vie stressants récents ou très anciens survenus pendant l'enfance) et extrinsèques, *i.e.* certaines pathologies chroniques liées à l'âge (troubles respiratoires, arythmie et insuffisance cardiaque, dyslipidémie, adiposité et troubles cognitifs). La dépression majeure, les phobies et les antécédents de TAG sont aussi des facteurs de risque indépendants de TAG (**chapitre 4**). Nos résultats suggèrent aussi pour la première fois que des variants des récepteurs adrénergiques alpha(1A) et beta2 sont des facteurs de risque de TAG du sujet âgé. Certains polymorphismes paraissent avoir un effet modérateur dans la réponse à des événements stressants récents ou anciens (interaction Gène x Environnement) (**chapitre 5**). Les limitations et les points forts de l'ensemble de ce travail sont détaillés en fin de section (**chapitre 6**).

## **SECTION 6 : CONCLUSIONS ET PERSPECTIVES**

### **Chapitre 1 : Conclusions**

Ces travaux indiquent que le TAG du sujet âgé est un trouble psychiatrique multifactoriel fréquent et distinct des autres troubles anxieux, impliquant à la fois des facteurs de risque très distaux (adversité pendant l'enfance) et d'autres plus proximaux (événements de vie récents et facteurs biologiques ou cliniques spécifiques du sujet âgé) et une vulnérabilité génétique liée aux récepteurs adrénergiques. Ils suggèrent en outre que le système adrénergique tout comme l'axe HHS, les deux grands systèmes physiologiques impliqués dans la réactivité au stress, seraient des facteurs étiopathogéniques majeurs du TAG.

### **Chapitre 2 : Perspectives**

Ce travail apporte de nouvelles connaissances sur les déterminants du TAG avec des implications cliniques en termes de diagnostic et d'étiologie. Il pourrait conduire à une meilleure compréhension des mécanismes physiopathologiques impliqués dans l'installation et le maintien du TAG avec à terme une possibilité de diagnostic plus précoce (biomarqueurs). L'identification de facteurs de risque modifiables du TAG pourrait permettre le développement de stratégies originales de prévention et d'intervention chez le sujet âgé. Les conséquences en santé publique pourraient être importantes puisque elles pourraient permettre une diminution du TAG mais aussi des pathologies comorbides, ainsi que de l'incapacité et de la mortalité qui lui sont associées. Ces effets pourraient être d'autant plus importants si l'on considère le vieillissement de la population et l'augmentation des troubles anxieux et du TAG attendue dans les années à venir (Wittchen et al., 2011).

## **SECTION 1 – INTRODUCTION**

## CHAPTER 1 – Epidemiology of anxiety disorders

### 1.1 Definitions and lifetime prevalence of anxiety disorders

Anxiety is a very common emotion and normal reaction to stress in the general population. Most of us feel anxious, when faced with big challenges such as making an important decision, performance test, interview, exam.... However, anxiety disorder is a frequent negative emotional state involving more than temporary worry or fear and characterized by excessive and persistent sense of apprehension, accompanied by specific somatic, cognitive, neurobiological and behavioral manifestations (Nuss, 2015). It can cause clinically significant distress or impairment in social, occupational, and other important areas of functioning and is notably characterized by intense fear, anxious arousal, irrational thought and avoidance. Until the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), anxiety disorder included GAD, phobia (social phobia, agoraphobia, and specific phobia), panic disorder, obsessive-compulsive disorder (OCD) and posttraumatic stress disorder (PTSD), but the two latter are no longer considered as anxiety disorders, whereas separation anxiety has been introduced for adults (American Psychiatric Association, 2000, 2013). The following section on general characteristics of anxiety disorder in terms of prevalence, comorbidity, disability, and global burden is based on large cohort or survey studies published before the 5<sup>th</sup> edition and thus according to the previous definition of anxiety disorders.

Approximately a fifth of people over 55 years of age have current mental disorders and nearly half reported mental disorder lifetime (Alonso et al., 2007; Kirmizioglu et al., 2009; Reynolds et al., 2015; Ritchie et al., 2004). Anxiety disorders are very common psychiatric disorders, even more so than depressive disorders, and according to large population-based surveys, between 5% and up to 31% of the population are affected by anxiety disorders during their lifetime (most frequently phobias and GAD) (**Table 1**). There are some variations notably due to methodological heterogeneity, regarding diagnostic criteria, *e.g.* use of different versions of the DSM or International Classification of Diseases (ICD), different diagnostic interview tools, *e.g.* the structured clinical interview for DSM (SCID), Diagnostic Interview Schedule (DIS), Composite International Diagnostic Interview (CIDI), The Mini International Neuropsychiatric Interview (MINI), as well as heterogeneity of target populations regarding age range, sex ratio, sampling procedure, and possibly cultural and ethnic differences (genetic or traumatic stressors influencing whole ethnic groups) (Steel et al., 2014; Volkert et al., 2013). In the French Esprit study, using MINI and DSM-IV criteria, 46% of community-dwelling elderly participants have experienced a mental disorder in their lifetime, a major depression for 26.5% and anxiety disorder for 29.4% (Ritchie et al., 2004). Phobias were the most frequently reported anxiety disorder (21.6% lifetime prevalence), GAD was relatively common (10.8%) whereas panic disorder, OCD, and PTSD were quite rare (less than 2%) (Ritchie et al., 2004).

**Table 1** Main large epidemiological community surveys of current and lifetime prevalence of common anxiety disorders<sup>1</sup>

Study name, Country (reference)	Number	Age range, Mean (SE)	Instrument & Diagnosis	Prevalence for anxiety disorders	
				Current (12 mo <sup>2</sup> )	Lifetime
GE  (Paulus et al., 2015)	1,342	4-7	DISYPS-II  DSM-IV	All anxiety disorders: 22.2%	
				GAD: 3.4%	
				Specific phobia: 9.8%; social phobia: 10.7%	
				Separation anxiety: 7%	
UG  (Abbo et al., 2013)	1,587	3-19	MINI-KID  DSM-IV- TR	All anxiety disorders: 26.6%	
				GAD: 1.4%	
				Specific phobia: 15.8%; social phobia: 5.2%; agoraphobia: 3.8%	
				Panic disorder: 3.0%	
NCS-A, USA  (Kessler et al., 2012a)	10,148	13-17	CIDI  DSM-IV	All anxiety disorders: 14.9% (1 mo); 24.9% (12 mo)	
				GAD: 0.4% (1 mo); 1.1% (12 mo)	
				Specific phobia: 9.5% (1 mo); 15.8% (12 mo)	
				Social phobia: 4.6% (1 mo); 8.2% (12 mo)	
				Agoraphobia: 0.8% (1 mo); 1.8% (12 mo)	
				Panic disorder: 0.8% (1 mo); 1.9% (12 mo)	
				Separation anxiety: 0.6% (1 mo); 1.6% (12 mo)	

SFS, IT (Faravelli et al., 2013)	2,363	14+	MINI FPI		All anxiety disorders: 12%
WMH, 17 countries worldwide (Kessler et al., 2007a)	85,052	16+	CIDI DSM-IV		All anxiety disorders: 4.8% (China) – 31% (USA)
NSMHW, AU (McEvoy et al., 2011)	8,841	16-85	CIDI DSM-IV	All anxiety disorders: 11.8%	All anxiety disorders: 20%
ECA, USA (Bourdon et al., 1992)	20,291	18+	NIMH-DIS DSM-III	All anxiety disorders (excluding GAD): 7.3% (1 mo); 8.9% (6 mo); 10.1% (12 mo) All phobias: 6.3% (1 mo); 7.7% (6 mo); 8.8% (12 mo) Panic disorder: 0.5% (1 mo); 0.8% (6 mo); 0.9% (12 mo)	All anxiety disorders (excluding GAD): 14.6% All phobias: 21.6% Panic disorder: 1.6%
NCS-R, USA (Kessler et al., 2005a; Kessler et al., 2005b)	9,282	18+	CIDI DSM-IV	All anxiety disorders: 18.1% GAD: 3.1% Specific phobia: 8.7%; social phobia: 6.8%; agoraphobia: 0.8% Panic disorder: 2.7% Separation anxiety: 0.9%	All anxiety disorders: 28.8% GAD: 5.7% Specific phobia: 12.5%; social phobia: 12.1%; agoraphobia: 1.4% Panic disorder: 4.7% Separation anxiety: 5.2%

ESEMeD, Europe (Alonso et al., 2007)	21,425	18+	CIDI DSM-IV	All anxiety disorders: 8.4%	All anxiety disorders: 14.5%
				GAD: 0.9%	GAD: 2.8%
				Specific phobia: 5.4%; social phobia: 1.6%; agoraphobia: 0.3%	Specific phobia: 8.3%; social phobia: 2.8%; agoraphobia: 0.8%
				Panic disorder: 0.7%	Panic disorder: 1.6%
MHGP, FR (Leray et al., 2011)	36,105	18+	MINI ICD-10	All anxiety disorders: 21.6% (6 mo)	
				GAD: 12.8% (6 mo)	
				Social phobia: 4.3%; agoraphobia: 2.1% (6 mo)	
BR (Mondin et al., 2013)	1,560	18-24	MINI DSM-IV	Panic disorder: 4.2% (6 mo)	
				All anxiety disorders: 20.9% (1 mo)	
				GAD: 9.7% (1 mo)	
				Social phobia: 4.0% (1 mo); agoraphobia: 12.3% (1 mo)	
KECAS, KR (Cho et al., 2015)	6,022	18-74	CIDI DSM-IV	Panic disorder: 2.5% (1 mo)	
				All anxiety disorders: 6.8%	All anxiety disorders: 8.7%
				GAD: 1.0%	GAD: 1.9%
				Specific phobia: 4.8%; social phobia: 0.3%; agoraphobia: 0.3%	Specific phobia: 5.2%; social phobia: 0.5%; agoraphobia: 0.4%
				Panic disorder: 0.3%	Panic disorder: 0.3%

WMHJS, JA (Ishikawa et al., 2015)	4,130	20+	CIDI DSM-IV	All anxiety disorders: 4.9% GAD: 1.3% Specific phobia: 2.3%	All anxiety disorders: 8.1% GAD: 2.6% Specific phobia: 3.4%
LASA, NL (Beekman et al., 1998)	3,107	55-85	DIS DSM-III	All anxiety disorders: 10.2% (6 mo) GAD: 7.3% (6 mo) All phobia: 3.1% (6 mo) Panic Disorder: 1.0% (6 mo)	
NESARC, USA (Reynolds et al., 2015)	12,312	55+	AUDADIS-IV DSM-IV	All anxiety disorders: 11.4% GAD: 2.8% Specific phobia: 5.8%; Social phobia: 1.5% Panic disorder: 1.4%	
NCS-R, USA (Byers et al., 2010)	2,575	55+	CIDI DSM-IV	All anxiety disorders: 11.6% GAD: 2.0% Specific phobia: 6.5%; Social phobia: 3.5%; agoraphobia: 0.8% Panic disorder: 1.3%	
NCS-R, USA (Gum et al., 2009)	1,461	65+	CIDI DSM-IV	All anxiety disorders: 7.0% GAD: 1.2% Specific phobia: 4.7%; Social phobia: 2.3%; agoraphobia: 0.4% Panic disorder: 0.7%	

ECA, USA (Regier et al., 1998)	5,702	65+	NIMH-DIS DSM-III	All anxiety disorders: 5.5% (1 mo) All types of phobias: 4.8% (1 mo) Panic disorder: 0.1% (1 mo)	
Esprit, FR (Ritchie et al., 2004)	1873	65+	MINI DSM-IV	All anxiety disorders: 14.2% (1 mo) GAD: 4.6% (6 mo) Agoraphobia and specific phobia: 10.1% (1 mo); Social phobia: 1.2% (1 mo) Panic disorder: 0.3% (1 mo)	All anxiety disorders: 29.4%; GAD: 10.8%; Agoraphobia and specific phobia: 17.6%; Social phobia: 6.0% Panic disorder: 2.0%
NMHWS, AU (Trollor et al., 2007b)	1792	65+	CIDI ICD-10	All anxiety disorders: 4.4% GAD: 2.4% Social phobia: 0.6%; agoraphobia: 0.8% Panic disorder: 0.8%	

<sup>1</sup>Including GAD, all types of phobias (specific phobia, social phobia, agoraphobia), panic disorder, and separation anxiety; <sup>2</sup>12 month prevalence (unless otherwise specified).

Country: AU=Australia; BR=Brazil; FR= France; GE=Germany; IT=Italian; JA=Japan; KR=South Korean; NL=Netherlands; UG= Uganda; USA=United States of America.

Instrument: AUDADIS-IV= the Alcohol Use Disorders and Associated Disabilities Interview Schedule IV; CIDI=Composite International Diagnostic Interview; DIS=Diagnostic Interview Schedule; DISYPS-II= Diagnostik-System für Psychische Ptorungen nach ICD-10 und DSM-IV für Kinder und Jugendliche-II; FPI= Florence Psychiatric Interview; K-SADS=the Schedule for Affective Disorders and Schizophrenia for School-Age Children lifetime Version; MINI=Mini-International Neuropsychiatric Interview; MINI-KID= MINI for children and adolescents; NIMH-DIS= the National Institute of Mental Health Diagnostic Interview Schedule.

Study name: ECA=Epidemiologic Catchment Area Program; ESEMeD=European Study of the Epidemiology of Mental Disorders; Esprit=Enquête de Santé Psychologique-Risques, Incidence et Traitement; KECAS= Korean Epidemiologic Catchment Area Study; LASA=Longitudinal Aging Study Amsterdam; MHGP= Mental Health in General Population; NCS-A= National Comorbidity Survey Adolescent Supplement; NCS-R= National Comorbidity Survey-Replication; NESARC= National Epidemiologic Survey on Alcohol and Related Conditions; NMHWS= National Mental Health and Well-being Survey; NSMHW=National Survey of Mental Health and Wellbeing; SFS= Sesto Fiorentino Study; WMH= World Mental Health Survey; WMHJS= World Mental Health Japan Survey.

## 1.2 Age of onset and course of anxiety disorders

Anxiety disorders can start in childhood, adolescence, or adulthood and current prevalence is relatively high throughout the life course. Late onset after the age of 60-65 is generally considered as uncommon (Jones, 2013; McEvoy et al., 2011; Roza et al., 2003) although a 34-year longitudinal Swedish cohort reported later age of onset (Samuelsson et al., 2005). The median age of onset is 11 years, specific phobia and separation anxiety disorder starting earliest (median age of onset around 7 years) and GAD latest (around 31 years) (Kessler et al., 2007a; Kessler et al., 2005a). A population-based study of 1,342 German children aged 4-7 years found a current prevalence of all anxiety disorders of 22.2%, consisting mainly of social phobia (10.7%), specific phobia (9.8%), and separation anxiety (7%) (Paulus et al., 2015), and very similar prevalence rates were observed in American or Ugandan children and adolescents (Abbo et al., 2013; Kessler et al., 2012a). In the longitudinal American Great Smoky Mountains Study, the 3-month prevalence of anxiety disorders was 2.4% among 1420 children aged 9 to 13 years and the cumulative prevalence (*i.e.* the accumulation of new cases in previously unaffected children) by 16 years of age was 9.9%. In 10,148 adolescents from the National Comorbidity Survey-Replication (NCS-R), anxiety disorders were the most common mental disorders, with a 12-month prevalence rate of 24.9% (Kessler et al., 2012a). A higher prevalence was found in some specific young populations, *e.g.* children with attention deficit hyperactivity disorder (ADHD) or with intellectual disability or in some non-Western countries (Abbo et al., 2013; Reardon et al., 2014; Shea et al., 2014; Vicente et al., 2012; Zarafshan et al., 2015).

In adults, a current prevalence rate around 10-20% is regularly reported in most countries (see Table 1); GAD and phobias are generally reported to be more frequent in adults, specific phobia and agoraphobias to be more common in adolescents (Bandelow et al., 2015a; Kessler et al., 2012b; Nguyen et al., 2013). In their review on the size and burden of mental disorders in Europe in 2010, Wittchen et al. estimated that each year 38.2% of the European Union population suffers from a mental disorder, anxiety disorders being the most frequent. The estimated 12-month prevalence for anxiety disorders is 14% compared with 6.9% and 5.4% for depression and dementia, respectively (Wittchen et al., 2011). This corresponds to 61.5 million of persons currently affected by anxiety disorders, compared with 30.3 for unipolar depression or 6.3 for dementias (Wittchen et al., 2011). In France, the large Mental Health in General Population (MHGP) survey (36,105 adults) reported an overall current prevalence of anxiety disorders of 21.6%, GAD being the most prevalent (12.8%), then social phobia (4.3%), panic disorder (4.2%) and agoraphobia (2.1%) with a trend for a decrease of all prevalence rates with age (between 18-34 years, 35-54 and 55 and more) (Leray et al., 2011).

## 1.3 Current prevalence rates of anxiety disorders in the elderly

Current prevalence rates for anxiety disorders in elderly cohort have been estimated to range between 4-14% in various community samples from Western countries (see Table 1) (Byers et al., 2010; Gum et al., 2009; Lenze et al., 2011b; Regier et al., 1998; Trollor et al., 2007b). In the French Esprit study, 14.2% of elderly participants were currently suffering from anxiety disorders (compared with 3.1% for major depression) (Ritchie et al., 2004). Very few studies have examined the oldest-old, but the prevalence could also be high, a prevalence rate of 9% has been reported in a Swedish sample of 338 non-demented 95-year olds (Borjesson-Hanson et al., 2011).

Although anxiety disorders are generally considered to be mostly chronic throughout the lifespan, the question remains as to whether anxiety remains persistent, increases or decreases in later life, some studies reporting stable, increased, or decreased rates with age (Blanchflower et al., 2008; Byers et al., 2010; Leray et al., 2011). One recent large study in primary health care in Qatar actually reported that the prevalence of anxiety disorders was higher in people aged 65 and over, than in the 50-64 year group (Bener et al., 2015). This is in agreement with a clinical study in patients diagnosed with anxiety in UK primary care which reported a U-shaped relationship with highest prevalence

among younger and older adults (Martin-Merino et al., 2010). In the U.S. National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) study, the lifetime prevalence of anxiety disorders was 11.4% and a U-shaped pattern was observed for the oldest-old (ages 85 and over) men but not women who showed a decreasing rate with age (Reynolds et al., 2015).

However, the prevalence rates for anxiety disorders in the elderly may be underestimated for several reasons, notably because patients with anxiety disorders are mostly treated as outpatients, and due to the difficulty to measure accurately anxiety in older adults. Indeed, elderly people as well as general participants and clinicians are more likely to misattribute anxiety symptoms to the normal aging process and to emphasize and treat their physical complaints and ignore the psychiatric symptoms. Some patients initially complain only of somatic symptoms before they are ultimately diagnosed with a primary anxiety disorder. Besides, older adults may minimize symptoms notably because of the stigma of mental illness. They also tend to manage and express their emotions differently to younger people. In addition, there are possible geographical or cultural differences. Furthermore, the clinical expression of anxiety disorders in old age may be different from that seen in younger age groups. Finally, a lower prevalence rate in the oldest old could result from the high comorbidity associated with anxiety disorder and subsequent increased mortality rate (mortality bias).

## 1.4 Comorbidity

Anxiety disorders not only have a high prevalence but are also frequently comorbid with somatic as well as other mental disorders (Leray et al., 2011). Patients with anxiety disorders frequently suffer from physical illness such as cardiovascular disease, hypertension, diabetes, asthma, and chronic pain (Gili et al., 2010; Hasan et al., 2015). Conversely, in clinical settings (*e.g.* patients with diabetes, cancer, cardiovascular, respiratory or neurological disease), the prevalence of anxiety is reported to be high, reaching over 50% (Lin et al., 2008; Linden et al., 2012; Tovilla-Zarate et al., 2012) and can contribute to the onset or increased severity of somatic diseases. It should be noted however, that the relative proportion of comorbid cases is usually higher than that found in representative population surveys, because individuals with two concomitant disorders, suffering from a high overall disease burden, are more likely to seek treatment than individuals with only one disorder.

Comorbidity with other psychiatric disorders is frequent in general populations and also greater in clinical settings (Kessler et al., 2007b; Leray et al., 2011; McEvoy et al., 2011; Schoevers et al., 2003). Overlap among anxiety disorders can be high, and in the NCS-R, high correlations were found between agoraphobia and social anxiety disorder, panic disorder or specific phobia; in addition, a high correlation was found between GAD and depressive disorder (Kessler et al., 2005b). Three Dutch studies reported variable rates of comorbidity between anxiety and depressive disorders. In the Netherlands Study of Depression and Anxiety (NESDA), 63% of the adults with current anxiety disorder were also comorbid with current depressive disorder, and 81% reported a lifetime depressive disorder (Lamers et al., 2011), whereas in the Rotterdam study, they were 11.6% and 49.3%, respectively (Hek et al., 2011). In the Longitudinal Aging Study Amsterdam (LASA) study, 26.1% of elderly people with anxiety disorders also met diagnostic criteria for major depression (Beekman et al., 2000). Very close rates were reported in two large population-based surveys; in 12,792 adults from the Canadian Community Health Survey Mental Health and Well-Being, 23% of those with anxiety disorders also met diagnostic criteria for major depressive disorder (Cairney et al., 2008); a comparable rate of 28.3% was found in the French MHGP survey of 36,105 adults (Leray et al., 2011). Comorbidity between anxiety and depressive disorder is also high in older adults with equivocal findings regarding the chronology of comorbid anxiety and mood disorders (Wolitzky-Taylor et al., 2010). Some authors suggest that depression and anxiety might not be discrete disorders in later life, representing instead phenotypical expressions of the same condition along a continuum (Schoevers et al., 2005).

## **1.5 Disability and mortality risk associated with anxiety disorders**

Anxiety disorders constitute a growing health problem world-wide which could affect the quality of life, limit the activities, and bring high public health burden (Wolitzky-Taylor et al., 2010). They are notably associated with impairment in cognitive functioning (understanding and communicating), social interaction, life activities and participation (joining in community activities) (Hendriks et al., 2014). The disability levels are particularly high for social anxiety disorder and mixed anxiety disorder (Hendriks et al., 2014). Anxiety disorders especially in the elderly are not only chronic and with high recurrence rates (Kessler et al., 2009), but also frequently accompanied with low compliance with medical treatment which also contribute to high disability level and thus an increased risk of mortality (Leray et al., 2011). Hence, anxiety disorders show substantial comorbidity with, and may be risk factor for, a number of psychiatric and somatic disorders as well as alcohol use disorders; this is also generally associated with greater severity and poorer health behaviors and can produce excess disability and increased use of general practice resources. As an example, both anxiety and depression disorders were reported to be associated with a higher disability level than having anxiety or depression alone (Prina et al., 2011).

The strength of the association between anxiety and disability increases with age. It is most likely bidirectional, anxiety being not only a consequence but also a predictor of disability in the elderly (Brenes et al., 2005; Brenes et al., 2008). However, very few prospective studies have focused on the diagnosis of anxiety disorder (rather than symptoms) and the effects of the main confounder (such as age, gender, depression, and medication) or mediators (chronic diseases) factors have rarely been considered (Lenze et al., 2011b). In the Esprit cohort, anxiety disorder was associated with an increased 7-year incidence of limitations in instrumental activities of daily living, independently of depression comorbidity and antidepressant or anxiolytic medication (Norton et al., 2012). Inconsistent data have been reported concerning anxiety disorder and mortality in elderly people but apart from the lack of consideration of confounding or mediating factors evoked above, this may depend on subtype of anxiety and severity (including comorbidity with depression). In the Esprit study, anxiety disorder was associated with all-cause mortality independently of depression, in elderly women only but not in men (Carrière et al., 2013).

## **1.6 The economic burden of anxiety disorders**

The high prevalence, chronicity, comorbidity, and associated disability contribute to the substantial economic burden of anxiety disorders on society worldwide (Baxter et al., 2014). Twelve years ago, the World Mental Health Survey reported that the public health burden of anxiety and depression ranked fourth among global burden of diseases, and would rapidly raise to the second (Kessler et al., 2004). More recently, Baxter et al. have calculated the global burden in 2010 of anxiety specifically, and estimated that anxiety disorders were the sixth leading cause of disability worldwide and accounted for 390 Disability-adjusted life years (DALYs) per 100,000 persons in 2010 (Baxter et al., 2014). For comparison, stroke, dementias and unipolar depression which are ranked fourth, second and first as the most important contributors to the burden of mental and neurological diseases accounted for 378, 537, and 103.7 DALYs, respectively (Wittchen et al., 2011). The costs of anxiety disorders (excluding PTSD) in Europe were estimated to be 41 billion Euros in 2004 and close to 66 billion Euros in 2010 (Baldwin et al., 2013). In U.S. the total cost ranged between 42-47 billion dollars, 54% being related to non-psychiatric medical treatment, 31% to psychiatric treatment and 10% to indirect costs such as work absenteeism and decrease in productivity (Greenberg et al., 1999). Increasing life expectancy will quickly result in higher demand for healthcare of the aging population. Indeed, according to the WHO, the proportion of the World's population over 60 years will increase from about 11% to 22% between 2000 and 2050, and the older people will outnumber children very soon (WHO, 2012).

In conclusion, anxiety disorders suffer from delayed detection, clinical misdiagnosis especially in the elderly, under or inappropriate treatment, and recurrence that also contribute to their economic costs. As one major contributor to the burden of disease worldwide, more research is urgently required into anxiety disorders to better understand the underlying pathophysiological mechanisms and find an effective way to improve prevention, recognition, treatment and develop novel interventions especially in older people. This is especially important for GAD, the most frequent anxiety disorder with phobia (Hek et al., 2011; Leray et al., 2011), which is frequently underdiagnosed and undertreated. This is notably due to the difficulty of recognizing clinical characteristics and physical manifestations of anxiety (which do not arise together in the form of an attack as in panic disorder, but rather in shifting combinations, as a more or less permanent state), as well as the lack of known specific initiating stimuli or stressors (in contrast with phobia or PTSD).

## CHAPTER 2 - Generalized anxiety disorder (GAD): general characteristics

### 2.1 Definitions and diagnosis criteria

GAD is a common psychiatric disorder currently characterized by persistent, excessive, and unrealistic worry about a number of events or activities, accompanied by psychic and somatic symptoms; the worry is difficult to control. It was first described by Sigmund Freud in 1894 as a generalized, persistent, and free-floating anxiety occurring frequently in the general population. However, this resembled normal anxiety and lacked distinguishing features compared to other anxiety disorders, so it had poor diagnostic reliability and validity. GAD as a residual category was first diagnosed only when no other diagnosis could be made on Axis I disorder in the Diagnostic and Statistical Manual of Mental Disorders, third edition (DSM-III) in 1980 (American Psychiatric Association, 1980). The period should last at least one month and the clinical manifestation included at least three of the following symptoms: motor tension, autonomic hyperactivity, apprehensive expectation increased, vigilance and scanning. However, GAD may be a prodromal or residual form of other disorders (Garvey et al., 1988; Uhde et al., 1985), and comorbidity with other psychiatric disorder prohibited the diagnosis of GAD according to the DSM-III. Therefore, the diagnostic criteria for GAD were revised in the DSM-III-R (American Psychiatric Association, 1987), GAD having an independent status with clear symptom criteria. Excessive and unrealistic worry regarding two or more life circumstances was identified as the core symptom, a criterion which was not present in the ICD, 10<sup>th</sup> revision (F.41.1) (WHO, 1992). ICD-10 places greater weight on somatic symptoms and focuses on physiological arousal such as trembling, sweating, palpitations as well as dizziness and explicitly limits comorbidity, excluding vigilance and scanning, and requires the patient has experienced at least 6 months with predominant tension, worry, and feelings of apprehension about everyday events and problems. In order to separate GAD from adjustment disorders, the duration of six months was also specified in the DSM-IV and for criterion A “unrealistic” was replaced by “excessive” worry and difficulty to control the worry was added (criterion B). For the association symptoms criterion C was revised to present 6 out of the 18 items forming the three headings of DSM-III-R, including motor tension, autonomic hyperactivity, as well as vigilance and scanning. With the publication of DSM-IV (American Psychiatric Association, 1994), GAD has been further refined to improve the discriminability and reliability (Di Nardo et al., 1993). Compared to DSM-III-R, DSM-IV removed the autonomic symptoms of anxiety, because these symptoms were less frequent than hyperarousal symptoms in GAD patients (Marten et al., 1993), and could bring diagnostic confusion with panic disorder. however, this modification did not consider the anxious patients in primary care settings who have predominant physical symptoms including autonomic symptoms (Rickels et al., 2001). The essential features remain excessive uncontrolled worry, with having multiple foci. In the text revision DSM-IV-TR, only prevalence in clinical settings and family pattern (*i.e.* evidence from twin studies that suggests a genetic contribution) has been updated. The fifth version of the DSM (DSM-5) retains the same diagnostic criteria for GAD as those of the DSM-IV-TR (American Psychiatric Association, 2013) (**Table 2**).

**Table 2** DSM-5 (or DSM-IV-TR) diagnostic criteria for GAD

<p>A. Excessive anxiety and worry (apprehensive expectation), occurring more days than not for at least 6 months, about a number of events or activities (such as work or school performance).</p> <p>B. The individual finds it difficult to control the worry.</p> <p>C. The anxiety and worry are associated with at least three of the following six symptoms (with at least some symptoms having been present for more days than not for the past 6 months).</p> <p>Note: Only one item is required in children.</p> <ul style="list-style-type: none"><li>(1) Restlessness or feeling keyed up or on edge.</li><li>(2) Being easily fatigued.</li><li>(3) Difficulty concentrating or mind going blank.</li><li>(4) Irritability.</li><li>(5) Muscle tension.</li><li>(6) Sleep disturbance (difficulty falling or staying asleep, or restless, unsatisfying sleep).</li></ul> <p>D. The anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.</p> <p>E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition (e.g., hyperthyroidism).</p> <p>F. The disturbance is not better explained by another mental disorder (e.g., anxiety or worry about having panic attacks in panic disorder, negative evaluation in social anxiety disorder [social phobia], contamination or other obsessions in obsessive-compulsive disorder, separation from attachment figures in separation anxiety disorder, reminders of traumatic events in posttraumatic stress disorder, gaining weight in anorexia nervosa, physical complaints in somatic symptom disorder, perceived appearance flaws in body dysmorphic disorder, having a serious illness in illness anxiety disorder, or the content of delusional beliefs in schizophrenia or delusional disorder).</p>
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## 2.2 GAD prevalence

Most epidemiological studies indicate that GAD is one of the most common anxiety disorders in adult general populations (Lenze et al., 2011b; Leray et al., 2011). Prevalence of GAD in general populations could notably depend on the diagnostic criteria (ICD-10, DSM III, DSM-IV or DSM-IV-TR) and instruments (using DIS, CIDI, or MINI), reference periods (1, 6, 12 months, or lifetime), as well as heterogeneity of study populations regarding age, sex, and possibly cultural and ethnic differences. In large epidemiological studies, lifetime prevalence was estimated to range between 1.9% (Muhsen et al., 2008) and 10.8% (Ritchie et al., 2004) and current prevalence between 0.4% (Kessler et al., 2012a) and 13.4% (Ansseau et al., 2008) (see above, Table 1 and below, Table 3 in chapter 5). The prevalence in special treatment settings is even higher (Ansseau et al., 2008; Milanak et al., 2013). In Europe, Wittchen et al. estimated that 8.9 million persons were currently suffering from GAD, the 12-month prevalence rate ranging between 2.2 and 4.3% [median (interquartile range, IQR)=2 (0.6-2.2)] (Wittchen et al., 2011). The large ESEMeD study, which included adults from Belgium, France, Germany, Italy, the Netherlands, and Spain, estimated the lifetime and 12-month prevalence of GAD at 2.8% and 0.9%, respectively (Alonso et al., 2007). A systematic review recently reported the prevalence for “subthreshold” GAD, defined by relaxing at least one of the diagnostic criteria from the standardized diagnostic manuals (DSM-III-R, DSM-IV or ICD-10), as being nearly twice as high as those for the full syndrome GAD in the general population as well as in specific patient populations (e.g. primary care or psychiatry) (Haller et al., 2014).

## 2.3 Age of onset and course

The current prevalence of GAD in children and adolescents ranges between 0.4 and 3.4% (Abbo et al., 2013; Kessler et al., 2012a; Kessler et al., 2012b; Paulus et al., 2015). GAD is not frequently diagnosed in its pure form, and it is often comorbid with other anxiety disorders, especially separation anxiety disorder as well as social phobia. The prevalence estimates of GAD tend to increase with age (Beesdo et al., 2009). Data from the Early Developmental Stages of Psychopathology (EDSP) study indicates few onsets in childhood, the core incidence period being in adolescence and young adulthood (Beesdo et al., 2010). GAD prevalence has been reported to be higher in adults, especially in women (Grant et al., 2009; Hoge et al., 2012). There are indications that GAD is a common anxiety disorder in older adults (Bryant et al., 2008; Leray et al., 2011; Mackenzie et al., 2011; Schoevers et al., 2003), with current prevalence of 1.2%-7.3% (Beekman et al., 2000; Gum et al., 2009; Reynolds et al., 2015; Ritchie et al., 2004; Schoevers et al., 2003) and a low rate of full remission (Bruce et al., 2005; Hoffman et al., 2008; Wittchen, 2002). Recent evidence also suggests higher rates (3.4%) among the elderly (65+), and considerably lower rates (1.7%) in the 14-65 group (Wittchen et al., 2011). Some studies however, report that GAD prevalence peaks in adults and then appears to decline in elderly people (Gonçalves et al., 2012; Grant et al., 2005; Kessler et al., 2005a; Trollor et al., 2007a), leading to the assumption that older cases would principally represent the continuing chronic course of an early-onset illness with very rare new onset in old age (American Psychiatric Association, 1994). Regarding subthreshold GAD, older adults seem to have a higher 12-month prevalence rate than other age groups (Carter et al., 2001).

It should be noted however, that the prevalence may be underestimated in the elderly, because the recognition of GAD in general practice is relatively low, especially in older adults (Parmentier et al., 2013). GAD is frequently undiagnosed and/or untreated in elderly people notably because of the focus of patients and practitioners on physical symptoms and the general assumption that high rates of anxiety are to be expected in elderly persons due to increased vulnerability (Parmentier et al., 2013). Stigmatization, cultural and generational differences may lead to symptom minimization. The high comorbidity with other chronic disorders including psychiatric disorders (see below), especially depression and other easily-identifiable anxiety disorders may also contribute to underestimation. Clinical expression of GAD in old age may also be different to that in younger ages but this has not been thoroughly examined. Indeed, GAD is understudied compared with other anxiety disorders, and there is a relative paucity of published research devoted to the characteristics and etiology of this disorder (Dugas et al., 2010), especially in the elderly population (Byrne et al., 2010).

## 2.4 Psychiatric comorbidity

GAD can be comorbid with a number of other Axis I and II conditions. In a large sample of 43,093 American adults, the prevalence of having at least one comorbid psychiatric disorder was 90.2% among the participants having reported lifetime GAD (4.1%); 46% of the GAD cases were comorbid with major depression and 57% with another anxiety disorder, mainly phobia and to a lesser extent panic disorder (Blanco et al., 2014). This study also suggests that GAD and major depression are different nosological entities, with distinct latent structures, clinical manifestations, and patterns of comorbidity (Blanco et al., 2014). The large French MHGP study reported a high comorbidity of current GAD with mood disorders, 21.8% of GAD cases also reporting major depression (Leray et al., 2011). In the Esprit study, 15.5% of the GAD cases were comorbid with major depression (Ritchie et al., 2004). The comorbidity rates are higher in clinical settings. Hoge *et al.* in a recent review reported that 29-62% of GAD patients were estimated to have major depression, other frequent comorbidities being social phobia (34%) and alcohol misuse (38%) (Hoge et al., 2012). GAD comorbidity with panic disorder is frequent in the clinical context and ranges from 18% to 49% (Brown et al., 2001; Bruce et al., 2001; Garyfallos et al., 1999). In contrast, PTSD and OCD are comorbid with GAD at relatively lower rates of 3-13% and 4-13%, respectively (Brown et al., 2001; Bruce et al., 2001; Garyfallos et al., 1999). Gender differences in comorbidity pattern have been reported; women are more likely to have

GAD comorbid with another anxiety disorder (except social phobia) or mood disorders (except bipolar disorder), and men are more likely to have GAD comorbid with substance use disorders (including alcohol and drug use disorders, as well as nicotine dependence) (Vesga-Lopez et al., 2008).

Comorbidity is associated with greater impairment, more treatment seeking, and worse outcome than among people with non-comorbid GAD. A meta-analysis examined the impact of both pure GAD and GAD comorbid with depression or other disorders on functioning and quality of life. While pure GAD was associated with disability and impaired quality of life, patients with comorbidity showed increased impairment overall and reduced likelihood of GAD remission despite a greater probability of seeking treatment (Hoffman et al., 2008). Indeed only 18% of elderly patients with pure GAD, compared to 28.3% of those with comorbid Axis I disorders, were reported to seek professional help for GAD in the past year (Mackenzie et al., 2011). Health-related quality of life is reduced among GAD patients, and the one year prevalence-based estimated cost of GAD in Europe is approximately double that of any anxiety disorder, being estimated at 1804 Euros per GAD patient in 2004 (Andlin-Sobocki et al., 2005). The cost is likely to increase and to be higher in patients with comorbidity (Andlin-Sobocki et al., 2005; Hoffman et al., 2008).

## CHAPTER 3 - Treatments of GAD

GAD is a distressing chronic illness and often accompanied by somatic anxiety symptoms, so most patients are not recognized in primary care (Bryant et al., 2008). A European patient survey of 21,425 non-institutionalized adults in Belgium, France, Germany, Italy, the Netherlands, and Spain showed that only 54.5% of patients with anxiety disorder had received adequate treatment whatever the type of health setting, but less than 25% of patients with anxiety or depression reported adequate treatment in primary care (Fernandez et al., 2007). In addition, the World Mental Health Survey conducted among 84,848 community-dwelling adults with anxiety, mood and substance disorders in 17 countries reported that the rate of 12-month use of mental health services in developed cities only ranged from 4.3% to 17.9% (Wang et al., 2007b). The main reason was that patients with anxiety disorder and with GAD in particular, sought treatment by general practitioners for somatic symptoms like restlessness, being easily fatigued, irritability, difficulty concentrating, dyssomnia and muscle tension rather than for worrying, especially the elderly who also suffer from chronic diseases. Hence, these patients tend to be under-detected, under-treated, or treated inappropriately and the rates of full remission tend to be low (Hoge et al., 2012).

In general, GAD is treated with pharmacotherapy, psychotherapy, or both, which may depend both on the patient's preference and tolerability regarding potential side-effects or contraindications and the expertise of the clinician, as well as on the severity of the symptoms (including comorbidity) and the lack of response to a given therapy. The most recent international and independent guidelines for pharmacotherapy of GAD indicate that the first-line treatment choices are antidepressants, notably selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs), and the anticonvulsant pregabalin (Bandelow et al., 2012). Regarding psychotherapy, Ayers et al. reviewed 17 studies that met criteria for evidence-based treatment and found that relaxation training, cognitive behavioral therapy (CBT), supportive therapy and cognitive therapy could be efficacious for anxiety symptoms and disorders, with CBT showing the most empirical support for late-life GAD (Ayers et al., 2007). A recent meta-analysis comparing the efficacy of these various treatments indicated that numerically, the combination of CBT and drugs could yield the highest pre-post effect sizes ( $d=6.04$ ,  $CI=3.71-8.37$ ), followed by medications alone (SSRIs  $d=3.48$ ,  $CI=3.18-3.78$ ; SNRIs  $d=2.47$ ,  $CI=2.09-2.84$ ; benzodiazepines  $d=2.75$ ,  $CI=2.47-3.03$ ; pregabalin  $d=2.66$ ,  $2.14-3.18$ ), and then CBT alone ( $d=1.81$ ,  $CI=1.47-2.15$ ), which was however comparable to pill placebo ( $d=1.85$ ,  $CI=1.61-2.09$ ) but better than psychological placebo for which no significant improvement was found ( $d=1.42$ ,  $CI=0.76-2.09$ ) (Bandelow et al., 2015b).

### 3.1 Psychotherapy

Psychotherapy is optioned for treatment of GAD with the advantage of being side-effect free. CBT is considered the gold standard psychotherapeutic treatment focusing on challenging cognitive biases through cognitive restructuring and behaviors through graded exposure, teaching the patients specific skills to rerun in their daily routine as well as relaxation training (Kaczurkin et al., 2015). Behavioral interventions are aimed to decrease maladaptive behaviors and increase adaptive ones, by modifying their antecedents and consequences and by behavioral practices that result in new learning. The cognitive interventions aim to modify maladaptive cognitions, self-statements or beliefs (Craske, 2010), but their efficacy is also contingent on the ability of the therapist, the severity of the disorder, and the length of therapy. The efficacy of individual CBT has been demonstrated in many randomized clinical trials (RCTs). A Cochrane meta-analysis performed in 2007 found that CBT could reduce anxiety symptoms of GAD after short-term treatment, and CBT was superior to placebo and to treatment as usual (Hunot et al., 2007). The result was further confirmed in a recent meta-analysis on the efficacy of treatments for anxiety disorders (Bandelow et al., 2015b). The combination of CBT and antidepressant medication may be particularly effective for depressed elderly with comorbid GAD. Maintenance medication can be highly protective to avoid relapse, and CBT to allow sustained

remission without requiring long-term pharmacotherapy (Wetherell et al., 2013), but with variable efficacy between age groups. Meta-analyses report that CBT obtains lower efficacy in older adults than working-age people (Bandelow et al., 2014; Gould et al., 2012) and efficacy of cognitive restructuring in comorbid or cognitively impaired elderly has also been questioned. An earlier study found that CBT could significantly improve worry, depressive symptoms, and general mental health for older patients with GAD in primary care, however without significantly reducing on the mean change in severity than usual care (Stanley et al., 2009). A meta-analysis in older adults (aged 55 years and older) reported similar significant improvements using pharmacological and psychotherapeutic therapy over control conditions (Goncalves et al., 2012). New approaches in CBT using the internet services and the telephone show considerable promise in the effective treatment of a range of mental disorders (Andrews et al., 2010). They can also help overcome barriers that older adults sometimes face such as mobility and transport, and increase the accessibility to mental health services for people living in underserved areas that lack trained geriatric therapists (Brenes et al., 2014), although the oldest old may have limited internet access. However, the effectiveness of internet-based CBT needs to be further compared to traditional CBT and is fraught with other medicolegal and ethical problems notably due to the lack of personal contact between the patient and therapist. Hence, internet-based therapy cannot to date be recommended.

Other psychological therapies have been evaluated, but less frequently than CBT, *e.g.* mindfulness and non-face-to face therapies, which generally fail to show significant effects on GAD. Relaxation appears to have a positive effect on GAD, but pre-post effect sizes are lower than those of studies using pill placebo (Bandelow et al., 2015b). Psychodynamic therapy hypothesizes that insecure relationships and conflict are at the core of psychological problems; however, it has rarely been used in GAD, and the current literature does not support the effectiveness of this therapy (Leichsenring et al., 2009; Salzer et al., 2011). There is also a lack of published studies to support other forms of therapy such as supportive therapy and interpersonal therapy.

### 3.2 Pharmacotherapy

Although medication is helpful for alleviating symptoms, there is no clear time frame for exactly when medicine should be considered. Previous studies indicate that the response rate of GAD to drug treatment lies between 44% and 81% (Bandelow et al., 2013), remission rates are relatively modest, and relapse rates considerable (Hershenberg et al., 2014). Some studies reveal that the combination of pharmacotherapy and CBT is the best approach for treating anxiety disorders (Bandelow et al., 2015b; Crits-Christoph et al., 2011; Mohatt et al., 2014). There are no clear treatment guidelines outlining an effective treatment for GAD, and each patient may have a different treatment response. So far, there are different types of medicines to treat GAD in the short-term and long-term, however, drug interactions and contraindications should be considered especially for older patients. Ageing is indeed frequently associated with polymedication and pharmacodynamics' alterations (decreased renal clearance, decreased hepatic metabolism and increase in elimination half-life) generating an increased risk of drug side-effects and interactions.

Antidepressants are generally the first line medical treatment option. SSRIs have been shown to be the best-tolerated medications, and response rates are significantly higher than for placebos (Bandelow et al., 2015b). This class of medications includes escitalopram (Lexapro), paroxetine (Paxil), and sertraline (Zoloft). These antidepressants have powerful anxiolytic properties with a broad spectrum of activity that may improve comorbid affective disorders and symptoms of anxiety (Baldwin et al., 2014). They are relatively safe and are found to be efficacious for the symptomatic treatment of GAD; they can be used in children and adolescents, and can also reduce psychic and somatic symptoms (Rynn et al., 2001). Their exact mechanism of action on GAD has yet to be determined. SSRIs inhibit the reuptake of serotonin and subsequent degradation of serotonin, which leads to an accumulation of serotonin in the synaptic cleft available to bind to postsynaptic receptor, and can lead to down-regulation of post-synaptic serotonin receptor. The anxiolytic effects may also

involve other neurotransmitter systems and mechanisms, including down-regulation of noradrenergic receptors, although with less affinity than SNRIs (Kavan et al., 2009). The advantage of SSRIs is their potential for long-term use without fear of intolerance or abuse, but they also have potentially troublesome adverse effects such as agitation, sleep disturbances, and nervousness which may arise at the beginning of treatment. These effects may impair patients' compliance, so benzodiazepines could also be used during the initial few weeks of treatment to counteract the adverse effects. All these medications should be started at low doses and gradually titrated up to the therapeutic levels to avoid an initial exacerbation of anxiety, especially in elderly patients.

SNRIs act both on serotonin and noradrenaline and are potent inhibitors of their re-uptake. In the meta-analysis performed in 2015 by Bandelow et al (Bandelow et al., 2015b) SNRIs were found to significantly improve GAD symptoms but the pre-post effect size was slightly lower than that of SSRIs. RCTs have shown the efficiency of two SNRIs, duloxetine and venlafaxine, for treating GAD symptoms (Bandelow et al., 2015b; Hartford et al., 2007; Nicolini et al., 2009) with venlafaxine showing a slightly higher effect size in the meta-analysis (Bandelow et al., 2015b). Duloxetine was found to be superior to placebo in reducing severity of GAD symptoms in children and adolescents (Strawn et al., 2014) as well as in adults and elderly with GAD (Alaka et al., 2014; Ball et al., 2015). Sleep disturbances, nausea, agitation could occur at the start of treatment, but the adverse effects are mild.

Pregabalin is a synthetic gamma-aminobutyric acid (GABA) analog with anticonvulsant, anxiolytic, and analgesic activities. It is also approved to be the first-line treatment of GAD in many countries. It acts as a calcium channel modulator, in binding to the  $\alpha_2\delta$  subunit of the voltage-gated calcium channel in the central nervous system. This reduces the intracellular availability of calcium that is required for membrane fusion and release of neurotransmitter into the synaptic cleft, and results in significant inhibition of the release of glutamate, noradrenaline, substance P, and calcitonin gene-related peptide (Micheva et al., 2006; Pande et al., 2003). Pregabalin allows a relatively rapid onset of action and appears to be well-tolerated as a long-term therapy option in adult patients with GAD (Baldwin et al., 2011; Pande et al., 2003; Rickels et al., 2005). Compare to SSRIs and SNRIs, pregabalin improves not only psychic symptoms, but also somatic symptoms of GAD (Holsboer-Trachsler et al., 2013). It appears efficacious in geriatric GAD. In short-term treatment, it does not appear to have the withdrawal symptoms associated with benzodiazepines (Pande et al., 2003) but dizziness and somnolence are frequently reported especially at the beginning of pregabalin treatment.

Benzodiazepines bind to the gamma sub-unit of the GABA-A receptor complex and facilitate GABA inhibitory effects by acting on a chloride ion channel (Zorumski et al., 1991). They are used for relieving acute anxiety on a short-term basis (up to 4 weeks), but concerns about benzodiazepine side-effects among elderly, including cognitive impairment, psychomotor impairment, excessive daytime sedation, instability of gait, falls, and fractures, have limited their use (Gray et al., 2006; Petrov et al., 2014; Wetherell et al., 2011). The risk/benefit ratio of these medications is poorer in older adults than in younger adults. The elderly should start at half the dose prescribed for younger patients and benzodiazepines should be considered rather as short-term adjuncts to treatment. Moreover, benzodiazepines are not effective on the depressive symptoms that often accompany GAD. Long-term use of benzodiazepines can lead to substance dependence, tolerance effects, as well as severe withdrawal symptoms when stopping this treatment.

Antiadrenergic agents are not only used for their anti-hypertensive properties, but also have anxiolytic properties (Hood et al., 2011). They are primarily adrenergic antagonists of adrenergic receptors (alpha or beta blockers) inhibiting postganglionic functioning of the sympathetic nervous system although certain compounds such as clonidine are adrenergic agonists acting presynaptically on the  $\alpha_2$  receptor. The most frequently used are beta-blockers such as propranolol, which act on beta adrenoreceptors (Shad et al., 2011). They can prevent stimulatory hormones such as adrenaline and noradrenaline from binding to their receptors, and then decrease the stress response and physical symptoms of anxiety. Beta blockers have been prescribed as single-dose agents, they can rapidly reduce the peripheral physical symptoms (*e.g.* palpitations and hand trembling) of anxiety within 30-

60 min, and are used for “performance anxiety” but do not affect the cognitive and emotional symptoms of anxiety (Farach et al., 2012).

In conclusion, the treatment of anxiety disorders in elderly people is complicated and generally requires long-term action and continuation several months after remission. GAD symptoms may be triggered by a number of factors, *e.g.* social isolation, stressful life events, cardio or cerebrovascular comorbidity, biological vulnerability, and the condition may be aggravated by increasing physical ill-health. Older people generally have chronic disorders and take several medications, and little is known about the effect of somatic comorbidity and potential drug interaction. In addition, polymedication can increase the risk and the fear of side effects and thus lead to lower compliance by the patients. Frequent follow-up could encourage adherence and allow monitoring treatment response. RCTs especially psychotherapy trials are generally short-term, and long-term recurrence-prevention trials are rare notably in the elderly. The literature on the treatment of late-life GAD is, however, limited in comparison with other age groups and other anxiety disorders. There is a lack not only of new better-adapted treatments for older adults but most of all of a better understanding of the mechanisms of onset and maintenance of GAD and its deleterious effects on the brain and physiologic health of elderly people (Lenze et al., 2011b).

## CHAPTER 4 - Neurobiology of GAD

Although a number of neurobiological alterations have been reported in anxiety disorders, studies aimed specifically at identifying the core correlates of neurobiological underpinnings of GAD are relatively rare. Furthermore, findings can be influenced by psychiatric comorbidity, especially depression and age-related neurophysiological alterations. Many of these neurobiological mechanisms point to the central role of hyper-reactivity and a fear of negative emotional shifts as well as the use of worry to prevent emotional contrasts that are perceived as unmanageable (Newman et al., 2013). Besides, certain characteristic features of GAD, *e.g.* hypervigilance, arousal, increased muscle tension, tremor and palpitations could indicate a dysfunctioning of stress response and alterations in the levels of hormones and neurotransmitters related to the psychobiological responses to stress. Some specific structural and functional modifications as well as alterations in neurotransmitter and neuroendocrine systems have been reported, mostly in case-control studies with GAD patients.

### 4.1 Neuroimaging structural and functional modifications

Studies in structural magnetic resonance imaging (MRI) suggest both white and grey matter abnormalities that may partly explain the impaired cognitive control of anxiety in GAD as well as excessive and persistent worrying, anticipatory anxiety, and emotion regulation (Brambilla et al., 2012; Schienle et al., 2011). The volume of gray matter in amygdala is shown to be increased in adult patients compared to controls (Etkin et al., 2009; Schienle et al., 2011), but not in older adults over the age of 60 (Milham et al., 2005; Mohlman et al., 2009; Strawn et al., 2013). Some studies in adult patients with GAD also report larger volumes of the dorsomedial prefrontal cortex (PFC) (Schienle et al., 2011) and decreased bilateral hypothalamus volumes (Terlevic et al., 2013). Lifetime GAD is also independently associated with a significant reduction in total hippocampal volume (Abdallah et al., 2013; Hettema et al., 2012). Many studies find that the changes of volume and functional patterns in GAD are more pronounced in the right cerebral hemisphere than in the left (Brambilla et al., 2012; Etkin et al., 2009; Schienle et al., 2011).

The amygdala is one of the most investigated brain structures in GAD. It seems to mediate all kinds of emotional stimuli, and fear in particular, and is comprised of multiple nuclei that are reciprocally connected to the hypothalamus, hippocampus, and neocortex-structures. Functional neuroimaging studies have helped progress in the understanding of these mechanisms and show that the emotional regulation as well as emotional conflicts are associated with the changing of activation of the amygdala (Blair et al., 2012; Etkin et al., 2010; Strawn et al., 2012). Childhood/adolescents studies show that GAD patients exhibited increased amygdala activation to threat stimuli (Monk et al., 2008; Nitschke et al., 2009). However, in adults GAD patients findings on amygdala activity are less consistent. Some studies have reported a heightened amygdala response to all stimuli (aversive or not) (Etkin et al., 2010; Nitschke et al., 2009), while others have found no group differences between GAD patients and control subjects or a reduced amygdala response to emotional stimuli (Blair et al., 2012; Whalen et al., 2008). Yassa et al. suggest that these discrepancies could be due to the quality of the emotional task used to examine the cerebral activity via functional imagery (Yassa et al., 2012). Indeed research in humans has mostly focused on the study of aversive responses to discrete cues using short-duration presentation of aversive stimuli. Actually, fear and anxiety may be expressed through two distinct and complementary systems, a rapid response system that mediates short-term responses to menacing stimuli and includes the amygdala, and a second system that includes the bed nucleus of the stria terminalis (BNST) and which continues to influence behavior long after the initiating stimulus has been terminated (Davis et al., 2010). Based on these observations, Yassa et al. have hypothesized that using a task inducing a state of sustained anxiety could allow to investigate amygdala activity during a prolonged stressful task, more consistent with stress of everyday life. They used a gambling game involving non-contingent monetary loss to explore the amygdala activity regarding anticipation in different conditions. Compared to controls there was decreased activation in

the bilateral amygdala in GAD patients and increased activity in the BNST, when comparing high uncertainty to low uncertainty conditions (Yassa et al., 2012). The authors thus suggest that GAD patients disengage the amygdala and its response to acute stress earlier than non-anxious controls, allowing the BNST to maintain a continuous anxious state, this process being more exaggerated in GAD patients compared to non-anxious individuals (Yassa et al., 2012).

There is also a lot of evidence including decreased connectivity between amygdala and the PFC and between the amygdala and the anterior cingulate cortex (ACC) in GAD patients compared to controls (Etkin et al., 2010; Etkin et al., 2009; Monk et al., 2008; Roy et al., 2013; Tromp et al., 2012). The PFC is crucial for emotional regulation, especially the ventromedial area which plays a pivotal role in modulating amygdala activity and controlling negative emotional responses (Diekhof et al., 2011). Furthermore increased ventromedial PFC activation has been reported after treatment with both psychotherapy and pharmacotherapy in young GAD patients (Maslowsky et al., 2010).

Recently, Andreescu et al. have explored the effect of age on neural network abnormalities observed in younger adults, by observing the impact of age and duration of illness on Default Mode Network (DMN) connectivity (Andreescu et al., 2014). The DMN is a functional network including several regions including the posterior cingulate cortex (PCC) and the medial PFC. This network shows a high level of functional connectivity at rest, its connectivity decreasing during activation tasks. The DMN is involved in multiple functions, notably emotional regulation. These authors have shown a significantly different pattern of connectivity in young vs. older GAD patients. Younger patients had significantly greater functional connectivity between the PCC and the medial PFC than the older participants. In addition worry severity in GAD patients was inversely correlated with the functional connectivity between the PCC and the medial PFC, suggesting that pathological worry, especially in the elderly, may be related to decreased flexibility in the prefrontal structures at rest (Andreescu et al., 2014).

Overall anxiety disorders are thought to result in part from disruption in the balance of activity in the emotional centers of the brain. In a healthy person, the frontal cortical regions regulate impulses, emotions, and behavior via inhibitory top-down control of emotional-processing structures. The most reliable finding is that GAD patients show persistent hyper-activation in the ACC and medial PFC during worry induction tasks compared to controls in adults (Paulesu et al., 2010) as well as in the elderly (Andreescu et al., 2011). Nevertheless, the results concerning worry suppression tasks are contradictory; the study by Ball et al. found a chronic over-responsiveness of limbic circuitry during emotional regulation tasks thus fatiguing the top-down system and rendering the PFC unable to effectively exert control when needed (Ball et al., 2013). Therefore, there is a hypo-activation of the cortical areas in GAD patients, leading to a deficit in the top-down control system (amygdala) during emotional regulation tasks (Ball et al., 2013; Mochcovitch et al., 2014). These results are confirmed in adolescent GAD patients who exhibit an elevated right amygdala response to viewing angry faces and this activation correlates positively with symptom severity. The overactivity in the right amygdala is correlated negatively with activity in the right ventrolateral PFC, also suggesting a deficiency in the top-down control system during emotional regulation task as a potential mechanism for elevated amygdala activity (Monk et al., 2008).

## **4.2 Neuroendocrine and neurotransmitter systems**

### **4.2.1 The hypothalamic-pituitary-adrenal axis**

The principal physiological responses to stress stimuli are mediated by the sympatho-adrenal system (sympathetic nervous system/adrenal medulla) and the hypothalamic-pituitary-adrenal (HPA) axis. Such responses are generated and coordinated in the brain. The HPA axis constitutes an important neuroendocrine response system which can coordinate the cross talk between the central nervous system and the endocrine system. In response to stress, corticotropin-releasing hormone

(CRH) and vasopressin are released by the hypothalamus in the portal circulation of the median eminence. They act on the pituitary gland to promote the secretion of adrenocorticotrophic hormone (ACTH) which in turn acts on the adrenal cortex leading to the synthesis and the secretion of glucocorticoid hormones (mainly cortisol in humans) (Faravelli et al., 2012). Once the stressor ends, circulating glucocorticoids act centrally on the brain via glucocorticoid receptors in order to induce negative feedback in the two main stress systems, the HPA system and the sympathetic system.

The stress response orchestrated by the HPA axis is a multisystem response, involving behavioral, physiological, and metabolic responses. These responses are initiated by the binding of cortisol on the glucocorticoid and the mineralocorticoid receptors. These two corticosteroid receptors have both genomic and non-genomic actions throughout the body and constitute major regulatory elements of the HPA axis (de Kloet et al., 2005; de Kloet et al., 2016).

Early life stress has been linked to chronically high levels of CRH as well as low cortisol levels (Heim et al., 2001). Cortisol is a major peripherally active stress hormone which has various effects on many tissues in the body. Chronic stress and sustained exposure to abnormal levels of cortisol can be harmful, leading to a number of physical (*e.g.* hypertension, immunosuppression, cardiovascular disease) and mental health problems (Feder et al., 2009). However, there is no unifying disturbance of HPA axis function across the affective disorders, meta-analyses generally reporting heightened cortisol levels in depression but lowered levels in PTSD (Belvederi Murri et al., 2014; Morris et al., 2012). Studies on HPA axis function in GAD are less common and mainly focus on clinical patients. They produce variable findings which may reflect differences in methodology regarding anxiety and stress assessments (basal state, during or after stress exposure or pharmacological challenge), cortisol circadian rhythm and time and condition of cortisol sampling (from plasma, saliva, urine, or hair), failure to consider potential moderators (including comorbidity) as well as heterogeneity regarding the nature, size, and age of the samples (Elnazer et al., 2014).

Despite some discrepancies in the literature, some evidence suggests that GAD is associated with cortisol hypersecretion and reduced negative feedback sensitivity, possibly due to chronic stress related to the inadequacy to cope with it or the perceived loss of controllability (see for review (Faravelli et al., 2012)). In older adults, cortisol levels were reported to be 40% to 60% higher in those with GAD than in non-anxious subjects (Lenze et al., 2011a; Mantella et al., 2008), and successful psychological or pharmacological treatment could reduce cortisol levels in subjects with GAD (Lenze et al., 2011b). An RCT with escitalopram in elderly GAD patients showed a 12 to 15% reduction in peak and improvement in anxiety symptomatology after 12 weeks of treatment (*vs.* no change with placebo) (Lenze et al., 2011a). They also showed that genetic variability in the serotonin transporter promoter was associated with cortisol changes during treatment; the patients with the “high-expressing allele”- group showing a reduction in cortisol levels, compared to no reduction in the “low-expressing allele”- group (Lenze et al., 2011a) (see Section 1, § 6.2.1). In the Esprit study of community-dwelling elderly, compared to mentally healthy participants, those with lifetime or current GAD showed increased levels of salivary diurnal cortisol (reaching a 55% increase for evening cortisol) and heightened reactivity under stress conditions but not basal conditions (Chaudieu et al., 2008). This pattern suggests a lower recuperation capacity after stress exposure and was distinct from that observed in depressed or trauma-exposed participants (Chaudieu et al., 2008).

The mechanisms involved in HPA axis dysfunction and their role in the pathophysiology of anxiety symptoms and syndromes remain to be further elucidated. A lack of downregulation of the system may be related to the changes in the sensitivity or in the number of CRH and/or glucocorticoid receptors of the hippocampus, limbic system, and cortical levels, which are brain areas associated with anxiety disorders (Faravelli et al., 2012; Ulrich-Lai et al., 2009). Increased cortisol level could increase the serotonin uptake and decrease the functional connectivity between amygdala and PFC as well as hippocampal volume, thus increasing the risk of GAD and reducing the emotion regulation abilities (Hilbert et al., 2014).

#### **4.2.2 Serotonin and GABA interplay**

Pharmacological interventions as well as investigations into neurotransmitter dysfunction in GAD have implicated an imbalance in the GABA, serotonergic and adrenergic systems. GABA is

the main inhibitory neurotransmitter in the brain, and GABAergic pathways are widely distributed in the central nervous system, including the hypothalamus, hippocampus, cerebral cortex and cerebellar cortex. The activity of GABAergic neurons is decreased in GAD. GABA-A receptor downregulation are reported in the temporal areas of the neocortex in GAD patients, and the GABA-A benzodiazepine receptor complex may play an important part in the etiology and mediation of GAD as suggested by the anxiolytic effects of benzodiazepines (see Section 1, § 3.2 Pharmacotherapy) (Nikolaus et al., 2010).

Serotonin neurons project widely in the brain and the serotonin transporter (5-HTT) is particularly concentrated in areas of dorsal and median raphe nuclei of the brainstem which are linked to anxiety. The activity of neurons innervating the PFC, basal ganglia and limbic region is decreased in GAD but not that of descending neurons from serotonergic nuclei in the brainstem and this altered neurotransmitter imbalance could contribute towards the feeling of anxiety associated with GAD (Jetty et al., 2001). Acute stress is also associated with increased serotonin turnover in several brain regions, including the amygdala, the nucleus accumbens and the PFC and was thought to be involved in mechanisms of GAD (Graeff et al., 2010).

### **4.2.3 The adrenergic/noradrenergic system**

Evidence for a pathological functioning of the adrenergic system in GAD is more limited and suggests an increased noradrenaline transmission from both locus coeruleus and the dorsal raphe nuclei (Berridge, 2008; Berridge et al., 2012). Animal models have shown that stress can cause a marked increase in noradrenaline release in several brain regions (especially in the hypothalamus, amygdala and locus coeruleus), which are closely related to the onset of anxiety and/or fear in animals exposed to stress (Tanaka et al., 2000). Stress also leads to the release of noradrenaline from brainstem nuclei, and most importantly from the locus coeruleus. This can result in an increased noradrenergic stimulation of numerous forebrain areas implicated in emotional behavior, such as the amygdala, the nucleus accumbens, the PFC and the hippocampus. Animal and human studies suggested chronic hyper-responsiveness of the locus coeruleus noradrenergic system is associated with anxiety disorders and cardiovascular problems, and blockade of  $\beta$ -adrenergic receptors in amygdala can oppose the development of aversive memories (Charney, 2003; McGaugh, 2004). The noradrenergic system is associated with behavioral inhibition, arousal and alert-waking states (Stone et al., 2011). Pathological dysfunctions of this system may be associated with GAD and mediate the autonomic symptoms associated with stress such as increased heart rate, tremor and sweating. An increase in plasma levels of noradrenaline as well as an increase of both heart rate and systolic blood pressure have been reported in adolescents with GAD after psychological stress compared with non-anxious participants, thus providing preliminary evidence of an hyperactivity of the noradrenergic system in response to stress in GAD (Gerra et al., 2000). The role of the adrenergic system in GAD is also supported by anxiolytic activity of antiadrenergic agents (see Section 1, § 3.2 Pharmacotherapy).

Together, hyperactive neurotransmitter circuits between the cortex, thalamus, amygdala and hypothalamus have been implicated in the disorder (Martin et al., 2009). Hypofunction of serotonergic neurons arising from the dorsal raphe nucleus and GABA-ergic neurons that are widely distributed in the brain may result in a lack of inhibitory effect (Dos Santos et al., 2005). Furthermore, overactivity of noradrenergic neurons arising from the locus coeruleus may produce excessive excitation in the brain areas implicated in GAD.

### **4.2.4 Adrenergic signaling and adrenergic receptors (ADRs)**

The adrenergic receptors (ADRs) are a class of G protein-coupled receptors and mediate action in the sympathetic nervous system through binding of catecholamines, essentially adrenaline and

noradrenaline as well as exogenous administered drugs, including alpha and beta antagonists (Dohlman et al., 1991). ADRs play a pivotal role in neuroendocrine communication and are involved in the neuroendocrine control of the stress response (Rajagopal et al., 2010). There are two main groups of ADRs alpha and beta, with several subtypes. The alpha1-ADRs signal mainly via G proteins of the  $G_{q/11}$  family coupled to activation of a phospholipase C and resulting in increased intracellular calcium. Conversely, alpha2-ADRs couple to the  $G_i$  proteins, which causes an inhibition of neurotransmitter release, as well as an inhibition of adenylate cyclase and subsequently of cAMP, resulting in smooth muscle contraction. On the other hand,  $\beta$ -ADRs mainly couple to  $G_s$  proteins (although  $\beta_2$  also couples to  $G_i$ ) to activate adenylate cyclase and cAMP production and notably, isoforms of some of the ion channels (Roskopf et al., 2008). Only a limited number of small studies have addressed the role of genetic variants in the signaling cascades of ADRs (Roskopf et al., 2008). This has rarely been examined in anxiety although pathological changes in the noradrenergic system have been reported notably in panic disorder, PTSD and GAD (Hilbert et al., 2014) in which alpha- and beta- blockers of ADRs could be effective in treating anxiety and physiological symptoms (Hood et al., 2011; Shad et al., 2011). To date, some genetic variants of *ADRs* have been reported to be associated with physiological stress response related to dysfunction of the adrenergic system (Freitas et al., 2008; Litonjua et al., 2010; Nielsen et al., 2016; Roskopf et al., 2008; Sober et al., 2009; Taylor, 2007). Significant associations were reported with certain psychiatric disorders *e.g.* schizophrenia (Clark et al., 2005; Liu et al., 2010; Lochman et al., 2013) and ADHD (Castro et al., 2013), although inconsistently (Elia et al., 2009; Huang et al., 2008), as well as PTSD, where an interacting effect with childhood trauma was recently reported (Liberzon et al., 2014). No significant associations were found with mood disorder (Burcescu et al., 2006; Ohara et al., 1998) and suicidal ideation (Perroud et al., 2009). Up to now, direct evidence for a role of *ADR* genetic variants in GAD is missing.

In conclusion, despite some increasing evidence for the involvement of structural and functional modifications of specific areas of the central nervous system as well as dysfunction in neurotransmitter and neuroendocrine systems in GAD, little is still known regarding the neurobiological bases of GAD especially in the elderly. Identifying the main processes involved could provide deeper understanding of the pathophysiological mechanisms implicated in the development and maintenance of GAD. One difficulty apart from recognizing clinical characteristics and physical manifestations of GAD remains however, to identify specific initiating stimuli or stressor and/or specific etiologic characteristics.

## CHAPTER 5 - Risk factors for GAD

Despite the urgent need to understand pathophysiological mechanisms to inform and prevent GAD especially in the elderly, only a small number of associated factors have been identified so far and the etiological causes remain in large part unknown. Many cross-sectional studies have examined the association between prevalent GAD and socio-demographic, lifestyle, biological, clinical and environment factors but mainly in younger populations (**Table 3**). However, especially for biological and clinical factors, cross-sectional designs preclude differentiating between factors that co-occur with, or result from, psychopathology and etiological factors related to the occurrence of GAD. Prospective longitudinal studies (especially those excluding prevalent GAD cases at baseline) can overcome the reverse causality bias and allow separating cause and effect in time and thus identify etiological factors (Moreno-Peral et al., 2014). However, few longitudinal studies have been performed on GAD incidence in the community setting (**Table 4**). The main categories of factors so far examined concern socio-demographic and lifestyle characteristics, physical health, as well as stressful environment and history of psychiatric disorder. The results are summarized below and the Tables 3 and 4 are at the end of this chapter. Genetic factors have also been investigated in distinct studies and will be discussed in the next part (see Section 1, §6.2 Candidate-gene studies of GAD).

### 5.1 Socio-demographic factors associated with GAD

**Age.** The relationship between age and prevalence of GAD appears to be complex and still a matter of debate (see Section 1, § 2.3 Age of onset and course). All cross-sectional studies in adult samples except one (Malard et al., 2015) report a higher risk in middle age groups (Goncalves et al., 2011; Grant et al., 2005; Leray et al., 2011; Lim et al., 2005; Mackenzie et al., 2011; Muhsen et al., 2008). This is also the case in the longitudinal NESARC study in adults (Grant et al., 2009), whereas the only two longitudinal studies in older adult groups fail to find significant associations with age (Chou et al., 2011; Schoevers et al., 2005).

**Gender.** Most previous cross-sectional and longitudinal studies in adults except one (Muhsen et al., 2008) report a risk of GAD twice as high in women than in men, irrespective of the age group (Ansseau et al., 2008; Grant et al., 2009; Grant et al., 2005; Leray et al., 2011; Lim et al., 2005; Mackenzie et al., 2011; Malard et al., 2015; Schoevers et al., 2003). On the other hand, in subjects aged 60 and over, the results are less consistent, non-significant associations being reported in cross-sectional studies (Beekman et al., 2000; Goncalves et al., 2011), but a significant association in the prospective NESARC study (Chou et al., 2011). In the AMSTEL study, Schoevers *et al.* report both significant and non-significant associations in cross-sectional (Schoevers et al., 2003) and longitudinal analyses (Schoevers et al., 2005), respectively.

**Ethnicity.** Although some studies find no significant differences in the prevalence rates between different ethnic groups (Chou et al., 2011; Mackenzie et al., 2011; Malard et al., 2015), others indicate significant differences (Carroll et al., 2011; Lim et al., 2005). In the large American NESARC study, Grant *et al.* report a higher GAD prevalence among White individuals relative to Black, Asian, or Hispanic individuals both in cross-sectional and longitudinal analyses (Grant et al., 2009; Grant et al., 2005).

**Education level.** Two studies in middle-age adults report a significant association between low level of education and prevalent GAD (Carroll et al., 2011; Muhsen et al., 2008). However, most cross-sectional and longitudinal studies fail to find significant associations (Chou et al., 2011; Grant et al., 2009; Grant et al., 2005; Lim et al., 2005; Mackenzie et al., 2011; Schoevers et al., 2003; Schoevers et al., 2005). Ansseau et al. also found no significant association but report a decreased GAD prevalence in adults with a university degree (Ansseau et al., 2008).

**Socio-economic status.** Low economic status, *e.g.* unemployment or low income, is generally associated with a higher risk of GAD in cross-sectional and longitudinal studies (Ansseau et al., 2008; Carroll et al., 2011; Grant et al., 2009; Grant et al., 2005; Leray et al., 2011; Mackenzie et al., 2011; Moffitt et al., 2007; Muhsen et al., 2008), but some studies in the elderly fail to find significant associations (Chou et al., 2011; Goncalves et al., 2011; Schoevers et al., 2003). In the Singapore NMHSA study, Lim *et al.* measured socio-economic status by both income level and type of housing, and observed that both the poorest and wealthiest income levels had higher rates of GAD (Lim et al., 2005). However, in a multivariate model, only the highest socio-economic group who live in a landed house (*i.e.* low rise/ low density residential housing) had a higher odd of GAD but the sample size was small, thus precluding definite conclusion (Lim et al., 2005).

**Marital status.** People living alone, divorced or widowed generally evidence higher rate of GAD compared to those who are married (Ansseau et al., 2008; Carroll et al., 2011; Grant et al., 2009; Grant et al., 2005; Lim et al., 2005; Mackenzie et al., 2011), although this seems not to be the case in the elderly (Chou et al., 2011; Goncalves et al., 2011; Schoevers et al., 2003; Schoevers et al., 2005).

## 5.2 Lifestyle characteristics

No significant associations are reported between prevalent GAD and weight, obesity or physical exercise (Carroll et al., 2011; Goncalves et al., 2011; Mackenzie et al., 2011; Muhsen et al., 2008) as well as smoking or alcohol use (Goncalves et al., 2011; Grant et al., 2005; Leray et al., 2011). Several cross-sectional studies report significant associations with drug, nicotine, or alcohol dependence (Grant et al., 2005; Leray et al., 2011), but this does not reach significance in longitudinal studies (Chou et al., 2011; Grant et al., 2009). This suggests that these associations could have resulted from or co-occurred with GAD, rather than being etiological factors.

## 5.3 Physical health

Few cross-sectional studies have examined specific physical illnesses as risk factors for GAD. Mackenzie *et al.* found that GAD was associated with various chronic health problems including hypertension or arteriosclerosis, cardiovascular disease, gastrointestinal disease, and arthritis. However, this failed to reach significance after controlling for socio-demographic characteristics, income and past-year psychiatric disorders (Mackenzie et al., 2011). Examining a large range of vascular factors, Lim et al. only found a significant association between prevalent GAD and coronary artery disease in adults but the low number of cases precluded definite conclusion (Lim et al., 2005). On the other hand, osteoporosis as well as respiratory disorders, *e.g.* poorer lung function, asthma, and chronic obstructive pulmonary disease were significantly associated with prevalent GAD in adults (Carroll et al., 2011; Lim et al., 2005; Muhsen et al., 2008) but this has not been examined in elderly cohorts.

In the ANSMHWP study, functional limitations but not overall chronic physical illness were significantly associated with increased risk of prevalent GAD in elderly (Goncalves et al., 2011). In the LASA study, there was no significant association between GAD prevalence and physical illnesses, functional limitations as well as cognitive decline (Beekman et al., 2000). In the AMSTEL study, number of chronic diseases as well as functional limitation and cognitive impairment were significantly associated with prevalent but not incident GAD over 3 years (Schoevers et al., 2003; Schoevers et al., 2005). One cannot thus exclude that chronic disorders could co-occur or be a consequence of GAD, rather than an etiological factor of GAD.

## 5.4 Stressful life events, parental characteristics, and history of psychiatric disorder

Stress in the environment can play an important role in both the development and manifestation of anxiety disorders throughout life. Early stressful events during childhood have consistently been found to be significantly associated with GAD in young and adult populations. Both retrospective cross-sectional and prospective studies have found that a history of child maltreatment, neglect, physical and sexual abuse, and childhood separation events (divorce/parental separation or losing a parent), were significantly associated with the development of GAD (Beesdo et al., 2010; Chou, 2012; Cougle et al., 2010; Kessler et al., 2008; Moffitt et al., 2007). Gender is reported to moderate the association between GAD and childhood physical and sexual abuse (Cougle et al., 2010), although not consistently (Chou, 2012). More proximal or recent life threatening events are also associated with prevalent GAD in adults (Lim et al., 2005; Muhsen et al., 2008). Only two cross-sectional studies have been performed in the elderly, a significant association with stressful events throughout life (early, midlife, or late-life) is reported in the LASA (Beekman et al., 2000), but not in the ANSMHWB study (Goncalves et al., 2011).

Parental characteristics, in terms of personality or history of mental disorders can also predict GAD in children. Several studies report that individuals with GAD are more likely to declare experiencing parental rejection or overprotection, maternal internalizing symptoms, reversed/enmeshed relationships with their mother, or greater current vulnerability in relation to their mother (Beesdo et al., 2010; Kessler et al., 2008; Moffitt et al., 2007). A parental history of GAD, other anxiety disorder, or major depression was also found to be associated with GAD in children as well as in adults (Beesdo et al., 2010; Goncalves et al., 2011; Kessler et al., 2008; Lieb et al., 2002; Moffitt et al., 2007).

Associations between prevalent GAD and other psychiatric disorders have frequently been reported in cross-sectional studies which can also reflect high comorbidity (Grant et al., 2005; Leray et al., 2011; Mackenzie et al., 2011; Schoevers et al., 2003). Some longitudinal studies also find that a personal history of psychiatric disorders (*e.g.* major depression, bipolar I disorder, social phobia, panic disorder, or PTSD) could increase the risk of incident GAD in adults as well as in the elderly (Chou et al., 2011; Grant et al., 2009; Schoevers et al., 2005).

In conclusion, most studies examining factors associated with GAD are cross-sectional which precludes differentiating direct causality from reverse causality. Longitudinal studies on GAD are rare and only two prospective studies have been performed specifically in the elderly. Both studies are limited by only one follow-up examination over 3 years and they focus mainly on sociodemographic characteristics and history of psychiatric disorder. The few candidate predictors for late-life GAD so far identified are being female and having a personal history of depression or anxiety disorder (Chou et al., 2011; Schoevers et al., 2005). Only one study examined chronic disorder globally with no significant association (Schoevers et al., 2005). The individual effect of otherwise frequent age-related chronic disorders (*e.g.* cardiovascular, respiratory or metabolic pathologies) has not been evaluated prospectively. Adverse environment could increase the risk of GAD in younger populations (Beesdo et al., 2010), but this has not been examined in the elderly although these age groups are more likely to accumulate stressful life events. Family history of mental disorder has not been examined in the elderly either, although it is associated with increased GAD in younger populations (Beesdo et al., 2010; Kessler et al., 2008). This could reflect both early shared environment and high heritability; an overlap of genetic risk factors in the case of comorbidity is also possible (Domschke et al., 2012).

**Table 3** Factors associated with prevalent GAD in large adult population-based studies

Study (reference)	Nb. of subjects	Age range or Mean (SE)]	Female (%)	Diagnosis	GAD prevalence	Significant associations (↑ or ↓)	Non-significant associations
NESARC, USA (Grant et al., 2005)	43,093	18+	n.s.	AUDADIS-IV DSM-IV	12-month: 2.1%; Lifetime: 4.1%	↓: Ethnicity (Asian, Hispanic or Black vs. White) ↑: Female, middle age (30-64), widowed, separated or divorced, low income, alcohol or nicotine dependence, other anxiety, mood, personality or drug use disorder	Education, Alcohol abuse, urbanicity, region of country
MHGP, FR (Leray et al., 2011)	36,105	18+	53.9%	MINI ICD-10	6-month: 12.8%	↑: Female, younger age (18-54), lower income, drug addiction, major depression	Marital status, employment status, alcohol abuse
GADIS II, BE&LU (Ansseau et al., 2008)	13,699	18+	60%	MINI DSM-IV	Point: 13.4%	↓: University degree, independent worker, retirement ↑: Female, age, region, living alone, unemployment	
NMHSA, SG (Lim et al., 2005)	2,847	20-59	63%	GHQ SCAN DSM-IV	1-month: 3%; Lifetime: 3.3%	↑: Female, age (45-59), married/divorced vs. single, ethnicity (Chinese vs. Malay), ≥ 3 physical comorbidities, chronic obstructive pulmonary disease, coronary artery disease, psychiatric comorbidity, recent life events, high socioeconomic group in landed house	Education, religion, heart or kidney failure, diabetes, hypertension, stroke, cancer, asthma, living alone, family history of mental illness
SIP, FR (Malard et al., 2015)	5,600	20-74	52.2%	MINI DSM-IV	6-month: 4.4%	↑: Female working in public sector	Age, origin, work status, work contract

INHis-1, IL (Muhsen et al., 2008)	2,082	21+ 48.3 (16.6)	54.6%	CIDI – Short Form	12-month: 1.8%; Lifetime 2.7%	↑: Middle age (40+), lower income, unemployment, low education, asthma, osteoporosis, life threatening events (bad accident, life threatening illness, being attacked...)	Gender, regular exercise, injury severe illness, rheumatic arthritis, hypertension, hyperlipidemia, past year death of family memb
Vietnam Experience Study, USA (Carroll et al., 2011)	4,256	31.1-49.0	0%	DIS DSM-III	12-month: 9.7%	↓: Married  ↑: Low education or income, White, smoking, alcohol consumption, have served in Vietnam, serious illness, poor lung function	Height, weight
NCS-R, USA (Cougles et al., 2010)	4,141	49.9 (16.4)	56%	CIDI DSM-IV	12-month: 8.4%	↑: History of physical or sexual abuse	
NESARC, USA (Mackenzie et al., 2011)	12,312	55+	59.9%	AUDADIS- IV DSM-IV	12-month: 2.8%	↑: Female, middle age (55-64), low income, divorced, widowed or separated, past year mood/anxiety disorder, cluster B (borderline, antisocial, narcissistic, histrionic) & C (avoidant, dependent, obsessive compulsive) personality disorder	Ethnicity, education, hypertension, arthritis, cardiovascular disease, high cholesterol, gastrointestinal disease, diabetes, any medical condition
ANSMHWB, AU (Goncalves et al., 2011)	3,035	55-85 68 (8)	52%	CIDI ICD-10 DSM-IV	12-month: 2.8%	↓: Older age (65-85 vs. 55-64) ↑: Functional limitations, worry about illness, family history of anxiety or depression	Gender, smoking, education, single, financial problem, family contact, self-assessed health, chronic illness, exercise, caregiver, stressful life events
LASA, NL (Beekman et al., 2000)	659	55-85	57.7%	CES-D first screening; NIMHDIS DSM-III	6-month: 7.3%	↑: Smaller network size, instrumental support, locus of control, life events (war), recent loss	Female, chronic physical illnesses, functional limitations, cognitive decline, early life events (parental loss, serious personal illness, neglect, and physical and sexual abuse)

AMSTEL, NL (Schoevers et al., 2003)	4,051	65-84; 74.4 (5.7)	62.4%	GMS- AGECAT	1-month 2.9%	↓: Unmarried  ↑: Female, personal history of depression or GAD, number of chronic disease, disability in Activities of Daily Living, cognitive impairment	Age, education, social or professional support, disability Instrumental Activities of Daily Living
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↑ increased risk; ↓ decreased risk; n.s. not specified.

#### ABBREVIATIONS:

Country: AU= Australia; BE=Belgium; FR= France; IL=Israel; LU=Luxemburg; NL=Netherlands; SG= Singapore; USA= United States.

Instrument: AUDADIS-IV= Alcohol Use Disorder and Associated Disabilities Interview Schedule IV; CES-D= Center for Epidemiologic Studies-Depression Scale; CIDI=Composite International Diagnostic Interview; DIS= Diagnostic Interview Schedule; GHQ=General Health Questionnaire; GMS-AGECAT=Geriatric Mental State Automated Geriatric Examination for Computer Assisted Taxonomy; MINI= Mini-International Neuropsychiatric Interview.

Study name: AMSTEL= Amsterdam Study of the Elderly; ANSMHWP=Australian National Survey of Mental Health and Well-Being; GADIS = Generalized Anxiety Disorder Impact Survey; INHIS-1=Israeli national health interview survey; LASA= Longitudinal Aging Study Amsterdam; MHGP=mental health in general population survey; NCS-R= National Comorbidity Survey-Replication; NESARC= the National Epidemiologic Survey on Alcohol and Related Conditions; NIMHDIS=National Institute of Mental Health Diagnostic Interview Schedule; NMHS(A)=the National Mental Survey (adults) of Singapore; SCAN=Schedule for Clinical Assessment in Neuropsychiatry; SIP=Sant  et Itin raire Professionnel.

**Table 4** Prospective studies having examined risk factors for incident GAD in general population

Study (reference)	Nb. of subjects	Age at baseline	Female (%)	Instrument Diagnosis	Follow-up; GAD incidence	Significant associations (↑ or ↓)	Other non-significant associations	Statistical model & adjustment factors
DMHDS, NZ  (Moffitt et al., 2007)	945	Birth	48%	DSM-III-R & DSM-IV at age 26 and over	Up to 32 years; Cumulative from age 18 to 32: 6%	↑: Mental health services or psychiatric medication (age 20-32), maternal internalizing symptoms, low socio- economic status, maltreatment, internalizing, or conduct problems before age 11, high negative emotionality (age 18)	Family history of depression or anxiety, lost a parent before age 11, depression symptom at age 9, self- esteem age 11-15, positive emotionality age 18	Bivariate regression/ sex
EDSP, GE (Beesdo et al., 2010)	3,021	14-24	49%	DIA-X/M- CIDI  DSM-IV	10-year; cumulative at age 34: 4.3%	↑: Parental GAD, depression, overprotection, family dysfunctioning, childhood separation, behavioral inhibition, reward dependence	Parent substance-use disorder, parental emotional warmth, novelty seeking, resilience	Cox regression/ Age, sex
NCS,USA (Kessler et al., 2008)	5,001	15-54	n.s. ~56%	CIDI  DSM-IV	10-year  3.6%	Childhood adversities (neglect, physical or sexual abuse, parent divorce), neuroticism, parent history of major depression, GAD, substance or panic disorder	Parental death, respondent personality (extroversion, openness to experience)	Discrete-time survival analysis / age at interview, gender, ethnicity
NESARC Wave 2, USA  (Grant et al., 2009)	34,653	18+	n.s.	AUDADIS -IV  DSM-IV	1-year;  1.12% (new cases)	↓: Hispanics vs. White; histrionic personality  ↑: Female, age (30-54), low income, separated/divorced/widowed, unipolar and bipolar disorder, dysthymia, phobia, PTSD, alcohol or drug disorder, personality disorder	Education, urbanicity, region	Multivariate logistic regression/socio- demographic factors and other psychiatric disorders

NESARC, USA (Chou et al., 2011)	8,012	60+	n.s. ~60%	AUDADIS -IV  DSM-IV	3-year;  1.63% (new cases)	↑: Female, history of PTSD, narcissistic personality	Age, marital status, ethnicity, education, income, mood disorder, other anxiety disorder (except PTSD), nicotine dependence, alcohol use disorder	Multivariate logistic regression/ Sociodemographic factors and other psychiatric disorder
AMSTEL, NL (Schoevers et al., 2005)	1,915	65-84	63.1%	GMS- AGECAT	3-year;  3.9% (new cases)	↑: Personal history of depression/anxiety, recent decrease in Instrumental Activities of Daily Living	Age, female, education, not married, social support, family history of mental illness, chronic illnesses, disability, cognitive impairment, new illness	Backward stepwise multivariate logistic regression

↑ increased risk; ↓ decreased risk; n.s. not specified.

#### ABBREVIATIONS:

Country: GE=Germany; NZ=New Zealand; NL=Netherlands; USA= United States of America

Instrument: DIA-X /M-CIDI= Munich-Composite International Diagnostic Interview; GMS-AGECAT=Geriatric Mental State Automated Geriatric Examination for Computer Assisted Taxonomy; MINI= Mini-International Neuropsychiatric Interview.

Study name: AMSTEL= Amsterdam Study of the Elderly; DMHDS= Dunedin Multidisciplinary Health and Development Study; EDSP= Early Developmental Stages of Psychopathology study; NCS=National Comorbidity Survey; NESARC= the National Epidemiologic Survey on Alcohol and Related Conditions.

## CHAPTER 6 - Heritability and genetic risk factors for GAD

The contribution of genetic factors to the etiology of a disease can be estimated by means of clinical genetic studies comprising family studies, twin studies, adoption studies and segregation studies. Molecular genetic studies, comprising linkage studies, association studies and genome-wide association studies (GWAS) can then serve to identify specific risk loci (linkage studies) or risk alleles in a candidate gene (association studies) or a hypothesis-free (GWAS) approach.

### 6.1 Heritability estimates: family and twin studies

Segregation studies suggest anxiety disorders to be complex genetic disorders more likely driven by an interaction of several vulnerability genes, each possibly conferring a relatively small effect, and environmental factors. A substantial role for genetic factors in the familial transmission of anxiety disorders has been reported, with heritability around 30% for GAD and PTSD, around 50% for panic disorder and for social phobia, and 67% for agoraphobia (Domschke et al., 2012; Hettema et al., 2001). An overlap between genetic risk factors for GAD, PTSD, and major depression has been suggested (Domschke et al., 2012). Family studies found up to a 3-times increased risk in first-degree relatives of patients with GAD (Hettema et al., 2001). A recent review also supported the familial aggregation of GAD and two family studies showed a significant association between GAD in the probands and in first-degree relatives, with a summary OR for both studies of 6.08 (95%CI: 2.5-14.9) (Shimada-Sugimoto et al., 2015). So far, few genetic associations have been examined for GAD specifically.

### 6.2 Candidate-gene studies of GAD

In the majority of genetic studies, genes are selected based on their known or perceived involvement in the aetiology, pathology, or neurobiology of the disease. Within these genes, single nucleotide polymorphisms (SNPs, single base-pair changes), or variable number of tandem repeats (VNTRs, *i.e.* different lengths of repeating sequence patterns) are generally chosen for analysis, favoring functionally relevant variants, *e.g.* influencing transcription factor binding, gene expression or protein levels or activity. Other variants have been selected based on prior studies which have shown that they were associated with other relevant phenotypes (*e.g.* biological systems or disorders). Association studies in anxiety disorders have investigated several hundred candidate genes so far, mostly selected based on results from animal studies, challenge experiments or psychopharmacological interventions in anxiety-related phenotypes or anxiety disorders. But few studies have been performed on GAD specifically, and thus a very limited number of genes have been directly implicated in GAD. To date, the most commonly studied genes in GAD are related to neurotransmitter systems (serotonin, dopamine), monoamine catabolism, neurotrophic factor, neuropeptides, as well as stress and HPA axis. These findings are shown in **Table 5** at the end of this chapter and are summarized in the next paragraphs. Variants are examined for their effect on GAD diagnosis or symptoms as well as treatment response in GAD patients (pharmacogenetic studies on SSRI or SNRI efficacy).

#### 6.2.1 Neurotransmitter signaling

##### *Serotoninerbic system*

The serotonin transporter (5-HTT) removes serotonin from the synapse, returning it to the

presynaptic neuron where the neurotransmitter can be degraded or released at a later time. It is probably the most studied candidate gene in psychiatry (Gatt et al., 2015; Karg et al., 2011). The transcriptional activity of this gene is modulated by a VNTR in the upstream regulatory region of the gene serotonin transporter polymorphism (5-HTTLPR), most commonly occurring as 14 repeats for the short (S) allele or as 16 repeats for the long (L) allele (44 bp deletion/insertion involving repeat elements 6 to 8). The short allele is known to show reduced transcriptional efficiency, decreased expression and serotonin transport activity, with therefore higher levels of synaptic serotonin, compared to the long allele (Karg et al., 2011). In depression, a recent meta-analysis indicated a significant interacting effect between the S allele and stressful life events, although a great number of methodologically sound studies report non-significant or even inverse interactions (with the L allele) suggesting other moderating factors and complex effects (Sharpley et al., 2014). For anxiety disorder, an association was found between 5-HTTLPR variants and OCD and, for PTSD, an interacting effect with traumatic events has also been reported (Gressier et al., 2013; Kolassa et al., 2010; Taylor, 2013; Wang et al., 2011). In GAD, two case-control studies have been performed, one in Polish Caucasians, which did not find significant associations (Samochowiec et al., 2004), and another in a Chinese sample which found a 2.3 fold increased risk of GAD in the patients with SS genotype compared with the other two genotypes (You et al., 2005). *Rs25531* is an A/G SNP located just upstream of 5-HTTLPR which has been reported to modify transcriptional activity of the gene. On the basis of these *in vitro* functional data, it has been proposed to recode the 5-HTTLPR/*rs25531* allele as S' or "low-expressing allele" (Sa, Lg) and L' or "high-expressing allele" (La haplotype). In their study on escitalopram response in elderly GAD patients, Lenze *et al.* divided the subjects into two groups, depending on whether they had no La haplotypes (La-, *i.e.* Sa/Sa or Lg/Sa or Lg/Lg alleles) or 1 or 2 La haplotypes (La+, *i.e.* La/La, or La/Sa, or La/Lg). Escitalopram had no efficacy in the La- group *vs.* moderate efficacy in the La+ group but this apparent genetic moderation of SSRI efficacy was actually due to a higher placebo response in La- compared to La+ subjects, suggesting a genetic effect on anxiety symptom variability unrelated to treatment rather than a pharmacodynamic effect (Lenze et al., 2010). In addition, Lenze *et al.* showed a reduction in cortisol levels in the La+ group compared to no reduction in the La- group but the difference failed to be significant due to small sample size (Lenze et al., 2011a). Lohoff *et al.* also reported that patients who were homozygous La/La showed better treatment response to the SNRI, venlafaxine, and remission compared with the S carriers (La/Lg or Lg/Lg) in European-American population (Lohoff et al., 2013b). In this sample, they also reported better treatment outcome over time in patients carrying the G allele of the serotonin receptor 2A gene (*HTR2A*) *rs7997012* as well as an interacting effect between 5-HTTLPR/*rs25531* haplotype and the *HTR2A* *rs7997012* SNP in the treatment response (Lohoff et al., 2013a). For GAD patients who carried the two "positive markers" for each gene (La/La + G/G or La/La + G/A), the antidepressant response was more efficient than in patients who did not (Lohoff et al., 2013b). No significant associations were found between another variant of *HTR2A* (*rs6313*) and GAD in German patients (Fehr et al., 2001).

Tryptophan hydroxylase (TPH) is an enzyme involved in the synthesis of serotonin (Hamon et al., 1981), which exists under two isoforms; TPH1 is expressed both in the periphery and central nervous system whereas TPH2 is only expressed in neuronal tissue. Functional polymorphisms involved in the regulation of brain serotonin synthesis have been identified. The *rs1800532* variant in the *TPH1* gene and located in intron 7 is another popular candidate gene in psychiatric disorder. However, no significant associations were found with GAD in German (Fehr et al., 2001) and Chinese patients (You et al., 2005). No *TPH2* variants have been examined in GAD.

### ***Dopaminergic system***

Genetic association studies examined the dopaminergic system given the important role of dopamine signaling in the stress response, dopamine being the precursor of noradrenaline and adrenaline (Arnsten, 2009). Genes involved in dopamine metabolism and signaling are also known to be involved in emotion regulation and dysregulation (Badgaiyan et al., 2009; Beiderbeck et al., 2012; Opmeer et al., 2010). A Finnish study examined the associations of 131 SNPs from 13 circadian-clock

genes or belonging to a signaling pathway that may connect circadian rhythmicity and anxiety with GAD or subthreshold cases. The only significant finding surviving Bonferroni correction concerned dopamine receptor D2 (*DRD2*) *rs4245146* and subthreshold GAD (Sipila et al., 2010). Nominally significant associations were also found with *DRD3* *rs11706283* and *rs7633291* (Sipila et al., 2010).

Regarding treatment response, no significant associations were found with two other *DRD2* SNPs (*rs1076560*, *rs1800497*) as well as a functional variant (*rs2550948*) in the dopamine active transporter 1 (*DAT1*) and venlafaxine treatment response in GAD (Saung et al., 2014). Perlis *et al.* examining 825 SNPs in 61 candidate genes (mainly related to neurotransmitter systems) reported no significant association for 10 other *DRD2* SNPs with duloxetine response and a nominally significant associations for four (*rs963468*, *rs167770*, *rs324023*, *rs324026*) of 29 *DRD3* variants in 259 White American patients with GAD (of whom 95 received placebo) (Perlis et al., 2013).

## 6.2.2 Neuropeptides

Neuropeptide Y (NPY) acts as a neurotransmitter in the autonomic nervous system and in brain circuits involved in anxiety. Recent evidence in humans suggests that NPY as an anxiolytic peptide could modulate stress reactions and emotional response (Zhou et al., 2008). *NPY* expression is notably high in the amygdala and hippocampus, areas of the limbic system that are involved in emotional responding (Heilig, 2004). The C allele of *rs16147* variant, located in the promoter region of *NPY* was associated with a 30% decrease in expression *in vivo* (Zhou et al., 2008). Amstadter *et al.* found that the TT homozygote of *rs16147* was associated with an increased risk of GAD after high stressor exposure in adults living in areas affected by the 2004 Florida Hurricanes (Amstadter et al., 2010).

## 6.2.3 Monoamine catabolism

The catechol-O-methyl-methyltransferase (COMT) gene, located on chromosome 22 between bands q11.1 and q11.2 (Grossman et al., 1992), codes for an  $Mg^{2+}$  dependent enzyme involved in the catabolism of catecholamines, including adrenaline, noradrenaline or dopamine, in the central nervous system and peripherally in red blood cells and liver (Lachman et al., 1996). It can play an important role in neuropsychiatric disorders. The most widely studied *COMT* variant is a functional SNP (*rs4680*) in the coding sequence of the gene, that leads to valine-to-methionine substitution at codon 158 (Val158Met). The Val allele leads to an increase in enzyme activity, which results in a decrease of cortical dopamine levels (Chen et al., 2004a). The enzyme activity is genetically polymorphic with a trimodal distribution (high activity in Val/Val genotype, intermediate activity in Val/Met genotype, and low activity in Met/Met genotype). A 3 to 4-fold difference in COMT activity is reported between Val/Val and Met/Met genotype (Hosak, 2007). Previous studies found a significant association between Met158 allele and anxiety in adolescent and adult populations (Lee et al., 2014; Olsson et al., 2005). A gene-environment interaction was also reported, homozygosity for the low-active Met allele and childhood adversities being associated with higher anxiety sensitivity, a personality trait which describes the fear of arousal-related sensations (Baumann et al., 2013). In GAD specifically, two studies failed to find significant associations with *rs4680* as well as two others SNPs (*rs2020917* and *rs737865*) (Hatzimanolis et al., 2013; Samochowiec et al., 2004). No significant associations were found between *rs4680* and antidepressant response to venlafaxine in GAD patients (Narasimhan et al., 2012) as well as between 55 other SNPs and response to duloxetine (Perlis et al., 2013), although two SNPs (*rs165774*, *rs165599*) were associated with anxiety improvement in the placebo group.

The monoamine oxidases (MAO) are enzymes that catalyze the oxidation of monoamines (catecholamines, serotonin), which exist in two forms: MAO-A and MAO-B. Both forms are bound to the outer membrane of mitochondria in most cell types in the body but show different specificities

(Edmondson et al., 2009). The MAO-A is located on chromosome Xp 11.4-p11.3 and transcriptional activity can be modulated by a 30-bp upstream VNTR in the promoter region. A greater frequency of the high activity (*MAOA-H*) alleles has been reported in female patients with GAD (Samochowiec et al., 2004). A study on a specific SNP in the *MAOA* gene (*rs6323*) which is in linkage disequilibrium with the upstream VNTR *MAOA* promoter gene polymorphism also found an over-representation of the T allele in GAD patients (Tadic et al., 2003). Perlis et al. found no significant association between 12 *MAOA* SNPs and response to duloxetine treatment in GAD (Perlis et al., 2013).

#### 6.2.4 Neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is a growth factor, member of the neurotrophin family which plays a key role in the formation, plasticity and integrity of neurons in brain circuits regulating emotion and has also been shown to regulate the HPA-axis stress response (Pan et al., 1998). Previous studies suggested BDNF may be a potential biomarker for psychiatric disorders (Bocchio-Chiavetto et al., 2010; Hashimoto et al., 2004; Ribases et al., 2004). Early life stress was shown to be associated with altered peripheral BDNF levels and anxiety in both rats and humans (Dalle Molle et al., 2012). The most frequently studied variant is the functional SNP *rs6265* in the coding region of the *BDNF* gene (Val66Met) which could affect intracellular distribution, packaging, and release of the BDNF protein *in vitro* (Egan et al., 2003). The Met allele has been associated with morphological changes in the hippocampus, PFC, and amygdale (Chen et al., 2004b; Egan et al., 2003; Koolschijn et al., 2010; Szeszko et al., 2005). A study in Brazil found that the Met allele was associated with an increased risk of GAD (Moreira et al., 2015). The Met allele was also associated with increased serum BDNF levels in GAD cases compared with the Val/Val genotype (Moreira et al., 2015). This was not confirmed in Chinese populations, the Val66Met genotype having no influence on GAD and BDNF levels, despite BDNF plasma level in GAD patients being lower than those in non-anxious controls (Wang et al., 2015). An interaction between *BDNF* Val66Met and stressful life events has been reported, the Met allele being at increased risk of negative emotionality in case of environmental adversity (Hayden et al., 2010; Perea et al., 2012) but this has not been examined in GAD. In addition, no significant association was found between *rs6265* and venlafaxine response in GAD patients (Narasimhan et al., 2011) although a meta-analysis reported that this SNP could influence response and tolerability to antidepressants in major depressive disorder (Kato et al., 2010). In addition, no significant associations were reported between 27 *BDNF* SNPs including *rs6265* and response to duloxetine (Perlis et al., 2013).

#### 6.2.5 HPA axis

Altered stress reactivity, *i.e.* hyperactivity of the HPA axis and hypersecretion of cortisol is likely to be involved in the pathogenesis of GAD (see above, Section 1, § 4.2.1). Some SNPs within single genes in the HPA axis have been individually associated with altered cortisol levels, or affective disorder (mainly depression or PTSD), or poor response to antidepressants (Derijk et al., 2008; Kumsta et al., 2007; Mehta et al., 2012; Papiol et al., 2007; van Zuiden et al., 2013; Velders et al., 2011; Zobel et al., 2010) and interacting effects with early stressful events have been reported (Zimmermann et al., 2011). Some polymorphisms in the corticotropin-releasing hormone receptor1 (*CRHR1*) gene have been associated with persistent hyperactivity of the HPA axis and may be associated with increased vulnerability to GAD (Gillespie et al., 2009). Mahon et al. reported interactions between trait anxiety and *CRHR1 rs7209436* and *rs110402* in association with basal cortisol levels, higher anxiety being associated with higher baseline cortisol only among individuals with the common homozygous genotype (Mahon et al., 2013). The FK506 binding protein 5 (FKBP5) is a co-chaperone of glucocorticoid receptor. Homozygous carriers of *FKBP5 rs3800373* and *rs4713916* were shown to be at risk of displaying chronically elevated cortisol levels after repeated psychosocial stress exposure, accompanied by increased self-report anxiety (Ising et al., 2008; Velders

et al., 2011). An increase in self-report anxiety after repeated stress exposure was also found for the *rs5522* variant of mineralocorticoid receptor (Nuclear Receptor Subfamily 3, Group C, Member 2, *NR3C2*) without however, an effect on cortisol. A *NR3C2* (*rs2070951*) by SSRI interaction has been reported, cortisol awakening response being only decreased in depressed patients using SSRI (Klok et al., 2011). In contrast, for glucocorticoid receptor (*NR3C1*) the *Bcl1* (*rs41423247*) variant (but not *rs6195*) was only associated with low anticipatory cortisol levels (Ising et al., 2008). One study reported that the haplotype of the *NR3C1* gene constituted of AA *rs6189/6190*, AA *rs6195*, and CC *rs41423247* could substantially modify the level of trait anxiety in asthmatic sufferers (Panek et al., 2014). Although likely, no genetic studies have been performed specifically on the risk of GAD associated with HPA-axis related genes.

Regarding treatment response in GAD, Perlis *et al.* among 825 SNPs in 61 candidate genes found two genetic variants of *CRHR1* (*rs12942254*, *rs4792888*) associated with duloxetine response in GAD patients and a marginal association with a *NR3C1* variant (*rs6196*) in the glucocorticoid receptor whereas no significant associations were found with four *FKBP5* variants (Perlis et al., 2013). A better treatment response to venlafaxine in GAD patients was also reported for one (*rs2856966*) of four variants of the pituitary adenylate cyclase-activating peptide (*PACAP*) gene, which is also known to influence HPA axis activity (Cooper et al., 2013a).

## 6.2.6 G protein-coupled receptor signaling

G-protein coupled receptors comprise one of the largest groups of signaling proteins; nearly 2% of our genes code for these receptors. Regulators of G-protein signaling (RGS) bind to G protein alpha subunits and increase their GTPase activity which then attenuates the downstream signaling (Hollinger et al., 2002). RGS2, a potent regulator that reduces G-protein activity, can selectively inhibit Gq alpha subunit (Heximer, 2004), therefore increasing GTPase activity. RGS2 was shown to bind selectively to the alpha1A ADR (*ADRA1A*) (Hague et al., 2005). A targeted genome screen in humans provided weak evidence of linkage between anxiety disorder proneness and markers flanking the *RGS2* gene (Smoller et al., 2001). The C allele of the functional *rs4606* variant was found to be associated with a twice-higher risk of GAD in adults exposed to the 2004 Florida hurricane (Koenen et al., 2009).

Genetic studies on the association between *ADR* variants and GAD are missing and no significant associations have been found between *ADRA1A*, alpha2A ADR (*ADRA2A*), and beta2 ADR (*ADRB2*) variants and duloxetine response in GAD patients (Perlis et al., 2013).

## 6.2.7 Estrogen receptors

Female gender is a risk factor for late-life anxiety and evidence suggests the involvement of estrogen, which can influence the levels of various neurotransmitters such as GABA and serotonin but also HPA axis and adrenergic system (Ancelin et al., 2005) which have been directly implicated in GAD (see above, Section 1, chapter 4 Neurobiology of GAD). Few studies however have examined the possibility that genetic variations in the estrogen receptors (ESR) could influence the risk of anxiety (Ryan et al., 2012). In the Esprit study, Ryan *et al.* examining two *ESR1* and three *ESR2* polymorphisms showed that the risk of GAD was twice as high in women (but not men) carrying the A allele of *ESR2 rs1256049* with no significant association with phobia (Ryan et al., 2011).

### 6.3 Genome-wide association studies (GWAS) of GAD

Unlike the candidate gene studies, which require *a priori* knowledge for gene selection, GWAS are “hypothesis-free” and could be a promising method for identification of common genetic variants allowing the genotyping of hundreds of thousands of polymorphisms across the whole genome. To date, the few GWAS performed on anxiety disorder have focused on phobias (Gelernter et al., 2003; Gelernter et al., 2004), PTSD (Logue et al., 2013; Xie et al., 2013), OCD (Stewart et al., 2013), and panic anxiety disorder (Otowa et al., 2012). No GWAS has taken into account the possibility of gene-environment interactions, and possible overlap in the heritability between psychiatric disorders, which could share genetic factors could constitute another difficulty. Besides, most GWAS of anxiety disorders have been limited by small sample sizes, with thus low overall power to detect significant associations (Otowa et al., 2016). Indeed, stringent criteria must be used to account for the multiple tests related to the huge number of SNPs which are analyzed individually, and most GWAS now employ a stringent p-value for significance threshold of  $5 \times 10^{-8}$ . The twin GWAS study on anxiety sensitivity showed an association with one SNP of genome-wide significance and with a further eight SNPs of near significance all occurring within the coding region of the binding protein, fox-1 homolog (*RBFOX1*) gene (Davies et al., 2015) but the etiological role of this locus remains undefined. This gene may have a critical role in GABAergic neuronal function which is altered in GAD patients. No GWAS has been performed on GAD specifically.

In conclusion, to date, genetic association and GWAS studies in anxiety disorder have frequently been size-limited, and have led to the identification of only a few variants which have also been frequently associated with other psychiatric disorders. Few genetic risk variants for GAD have been identified to date through studies of candidate genes mainly involved in neurotransmitter systems or neurogenesis (*5-HTTLPR*, *MAO-A*, *NPY*, *BDNF*, *DRD2*, *DRD3*, and *RGS2*). In all of them except in the Finnish case-control study (Sipila et al., 2010), only one variant for a given gene was examined and none of these findings have been replicated. Most genetic studies performed on GAD were case control studies and focused on young adults. It thus appears difficult to identify the main effects of common variants in GAD, and gene-gene and gene-environment interactions which may also play an important role, have not been examined. Candidate gene studies using a targeted strategy driven by pathobiochemical mechanisms could be better suited for hypothesis-testing regarding the specific involvement of genes susceptible to play a key role in the disease etiology and to assess some complex interactions. A few small genome-wide studies taking into account epigenetic factors (Epigenome-Wide Association Studies, EWAS) are now being performed on depression and PTSD which may be useful to disentangle the interactive effects of genetic and environmental factors conferring risk and resilience. To date, no GWAS or EWAS have been performed on GAD.

**Table 5** Candidate-gene studies on GAD

Reference	N	Ethnicity	Age range or mean (SD)	Diagnosis	Gene	Gene variant	Risk for GAD	Treatment response in GAD (antidepressant)
(Samochowiec et al., 2004)	63 GAD, 202 CTRL	CAU	39 (12) vs. 36 (14)	CIDI ICD-10	<i>5-HTT</i>	<i>5-HTTLPR</i>	NS	
					<i>COMT</i>	<i>rs4680</i>	NS	
					<i>MAO-A</i>	upstream VNTR	↑ for N >3 repeat alleles in women	
(You et al., 2005)	138 GAD, 90 CTRL	Asian (Han Chinese)	33.8 (14.1)	SCID DSM-IV	<i>5-HTT</i>	<i>5-HTTLPR</i>	↑ in SS	
					“	<i>VNTR in intron 2</i>	NS	
					<i>TPH1</i>	<i>rs1800532</i>	NS	
(Lenze et al., 2010)	125 GAD: 59 escitalopram, 66 placebo	CAU	60+	HAMA DSM-IV	<i>5-HTT</i>	<i>5-HTTLPR/rs25531</i>  ( <i>La, Lg, Sa</i> <i>haplotype</i> )		↑ in La+ group (La/La, La/Sa or La/Lg (escitalopram))
(Lohoff et al., 2013b)	112 GAD	EA	18+	HAMA CGI-S DSM-IV	<i>5-HTT</i>  x  <i>HTR2A</i>	<i>5-HTTLPR/rs25531</i> ( <i>La, Lg or S</i> ) x <i>rs7997012 (G/A)</i> <i>haplotype</i>		↑ in La/La or/and G allele [single marker: <i>5-HTT</i> haplotype or <i>HTR2A</i> SNP, or interaction] (venlafaxine)
(Lohoff et al., 2013a)	156 GAD	EA: 71.8%; AA: 26.3%; Other: 1.9%	18-85	HAMA DSM-IV	<i>HTR2A</i>	<i>rs7997012</i>		↑ in G allele (venlafaxine)
(Fehr et al., 2001)	50 GAD, 87 CTRL	CAU	45 (11.7)	CIDI	<i>HTR2A</i>	<i>rs6313</i>	NS	
				DSM-IV	<i>TPH1</i>	<i>rs1800532</i>	NS	

(Sipila et al., 2010)	73 GAD, 146 CTRL (or 103 subthreshold GAD, 206 CTRL)	Finnish	30+	CIDI DSM-IV	<i>DRD2</i>	<i>rs4245146</i>	↑ in C allele	
					"	<i>14 other SNPs</i>	NS	
					<i>DRD3</i>	<i>rs11706283</i>	↑ in C allele	
					"	<i>rs7633291</i>	↑ in T allele	
					"	<i>5 other SNPs</i>	NS	
					<i>Others</i>	<i>130 SNPs (11 genes)</i>	NS	
(Saung et al., 2014)	156 GAD	EA: 71.8%; AA: 26.3%; Other: 1.9%	18+	HAMA	<i>DRD2</i>	<i>rs1076560</i>	All NS (venlafaxine)	
				CGI-S	"	<i>rs1800497</i>		
				DSM-IV	<i>DAT1</i>	<i>rs2550948</i>		
(Amstadter et al., 2010)	616 (GAD prevalence 6.7%)	White: 90.6% Other: AA, Hispanics, and Asians	60+	SCID-IV			↑ in T allele	
				DSM-IV	<i>NPY</i>	<i>rs16147</i>	(after high hurricane exposure)	
(Hatzimanolis et al., 2013)	391 women (lifetime GAD 13%)	CAU	18+	MINI DSM-IV	<i>COMT</i>	<i>rs2020917</i> <i>rs737865, rs4680</i>	All NS	
(Narasimhan et al., 2012)	156 GAD	EA: 71.8%; AA: 26.3%; Other: 1.9%	18+	SCID HAMA DSM-IV	<i>COMT</i>	<i>rs4680</i>	NS (venlafaxine)	
(Tadic et al., 2003)	50 GAD 276 CTRL	CAU	Mean: 43(12)	MINI HAMA	<i>MAOA</i>	<i>rs6323</i>	↑ in T allele	

(Moreira et al., 2015)	121 GAD, 695 CTRL	Multiethnic, Brazil	18-35	MINI DSM-IV	<i>BDNF</i>	<i>rs6265</i>	↑ in Met allele
(Wang et al., 2015)	108 GAD, 99 CTRL	Asian (Han Chinese)	16-64	MINI HAMA	<i>BDNF</i>	<i>rs6265</i>	NS
(Narasimhan et al., 2011)	155 GAD	EA: 71.6%; AA: 26.5%; Other: 1.9%	18+	SCID HAMA DSM-IV	<i>BDNF</i>	<i>rs6265</i>	NS (venlafaxine)
(Cooper et al., 2013a)	109 GAD	EA	18+	HAMA CGI-I DSM-IV	<i>PACAP</i>	<i>rs2856966</i>	<i>rs2856966</i> : ↑ in allele A
					“	<i>rs928978</i>	(venlafaxine)
					“	<i>rs1610037</i>	
					“	<i>rs1893154</i>	
					“	<i>rs2231187</i>	Other <i>PACAP</i> SNPs: NS
					“	<i>rs2846811</i>	
					“	<i>rs8192595</i>	
					<i>PAC1</i>	<i>rs2267735</i>	NS
(Koenen et al., 2009)	607 (GAD prevalence 6.8%)	White: 90.6% Other: AA, Hispanics, and Asians	60+	SCID-IV DSM-IV	<i>RGS2</i>	<i>rs4606</i>	↑ in C allele (after hurricane)

(Cooper et al., 2013b)	156 GAD	EA: 71.8%	18+	HAMA	<i>OPRM1</i>	<i>rs1799971</i>	NS (venlafaxine)
		AA: 25% Other: 3.2%					
(Ryan et al., 2011)	1092 women (GAD prevalence 8%)	CAU	65+	MINI DSM-IV	<i>ESR1</i>	<i>rs2234693</i>	NS
					“	<i>rs9340799</i>	NS
					<i>ESR2</i>	<i>rs1256049</i>	↑in A allele
						<i>rs486938</i>	NS
						<i>rs1271572</i>	NS

↑ indicated increased risk of GAD compared to controls (CTRL) or an improvement of treatment response; NS: non-significant.

#### ABBREVIATIONS:

Ethnicity: AA=African-Americans; CAU=Caucasians; EA=European-Americans

Instrument: CGI-I =Clinical global impression of improvement; CGI-S = Clinical global impressions-severity; HADS-A =hospital anxiety and depression scale-anxiety; HAMA =Hamilton anxiety rating scale; MINI =Mini-International Neuropsychiatric Interview; SCID =structured clinical interview for DSM disorders; SCID-IV = structured clinical interview for DSM-IV.

## **SECTION 2 - THESIS HYPOTHESES AND OBJECTIVES**

GAD is a common mental disorder in the elderly, and a source of substantial socioeconomic and public health burden. It is, however, frequently under-recognized and misdiagnosed and thus under-treated. GAD is commonly considered as relatively chronic across the lifespan, with primary differences across age groups being mainly related to the content of an individual's worry during life. It is also sometimes considered as a simple marker of severity of other anxiety disorders or depression. To date few epidemiological studies have examined the risk factors for GAD in general populations, especially in the elderly. The overall goal of this thesis was to provide a deeper understanding of the clinical characteristics and risk factors for GAD in late life.

In light of the current literature reviewed above, our main hypotheses are that GAD in the elderly has characteristics distinct from other anxiety or mood disorders and could also show specific age-related predictors, which are different to those of younger age groups. Another hypothesis is that stressful life environment could be an important determinant of GAD in the elderly.

This study draws on data from the Esprit study, which is a prospective population-based study of neuropsychiatric disorders in community-dwelling French elderly people. It estimates the prevalence and incidence rates of GAD in elderly people focusing on biological and environmental factors as well as genetic vulnerability. It is hoped that this work could help in better identifying and treating GAD in the elderly, with thus major health consequences notably by decreasing comorbidity, disability, and mortality.

The three specific aims of this work are as follows:

**AIM 1:** to estimate the current and lifetime prevalence of GAD and describe the characteristics of subjects with GAD, notably focusing on associated socio-demographic, environmental, and biological factors as well as comorbidity with other psychiatric disorders in a large community-dwelling French elderly people.

**AIM 2:** to estimate the 12-year incident rate and predictors of incident GAD in elderly people among a large range of socio-demographic, lifestyle, environmental, biological, and clinical factors.

**AIM 3:** to determine the association between GAD and *ADRA* genetic variants in adrenergic-related pathways, and examine possible gene-environment interactions with stressful life events.

### **SECTION 3- SUBJECTS & METHODS**

## CHAPTER 1 - The Esprit study

### 1.1 Study design and population

The Esprit study is an ongoing longitudinal study which aims to determine biological, environmental and genetic risk factors for psychiatric disorders in elderly community-dwelling people (Ritchie et al., 2004). Eligible participants were non-institutionalized people aged 65 years or over, who were recruited by random selection from the fifteen electoral rolls of the Montpellier district between March 1999 and February 2001. The study is described in detail below and summarized in **Figure 1** (see next page).

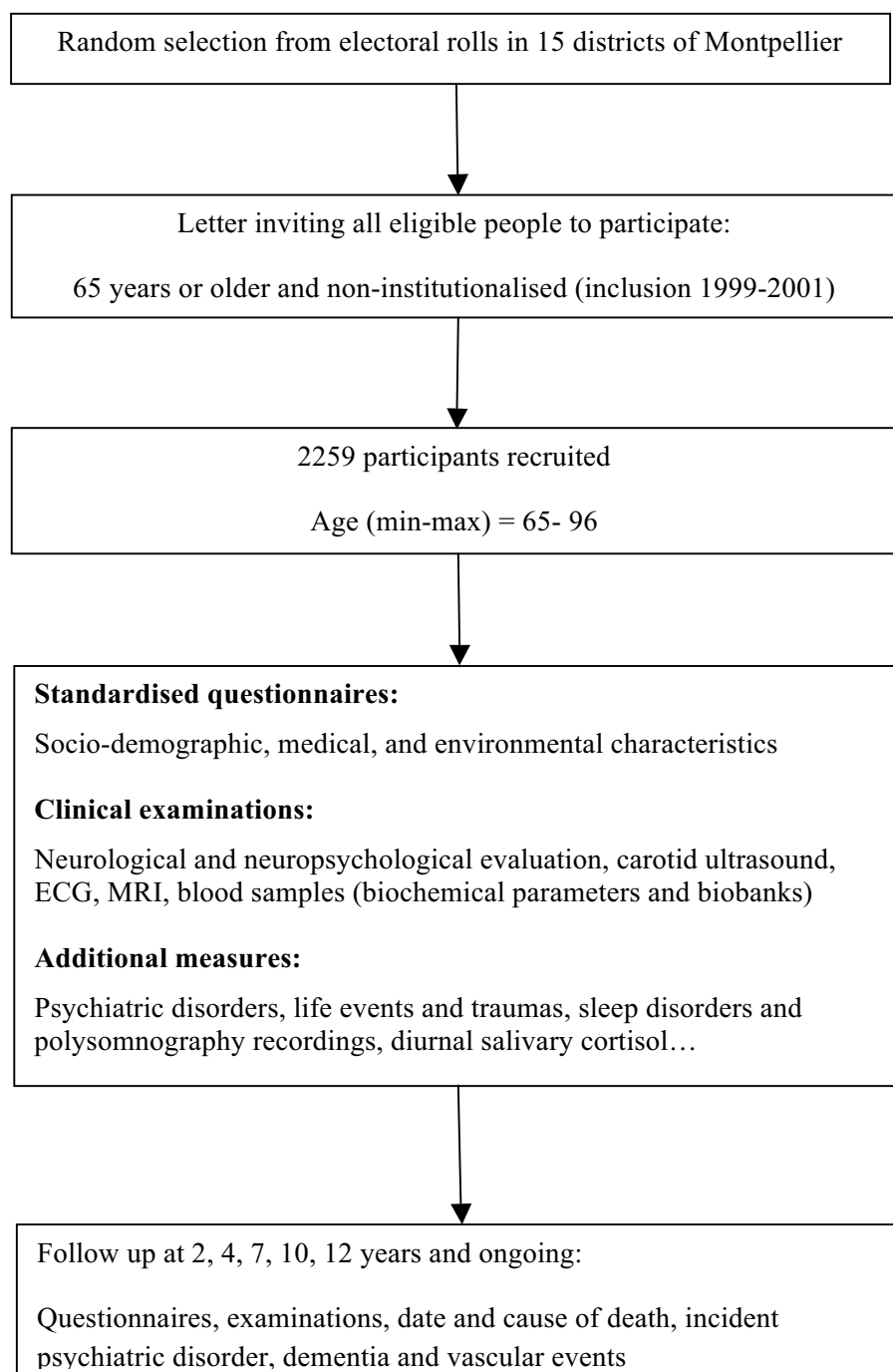
Randomly selected eligible persons were contacted by mail with a short letter explaining the study protocol and asking if they would like to participate in the study. They were then required to submit a form giving either their acceptance or refusal. If the person did not give any response, an attempt was made to contact the person by telephone. Twenty-four percent of eligible inhabitants did not respond to the letter and could not be contacted by phone. Of the persons contacted, 73% agreed to participate in the study. Those who refused to participate (of these, 3.3% were excluded owing to severe disability) were replaced by another subject drawn at random from the same electoral area, so that each division was equally represented. Subjects refusing were slightly older and more likely to live alone than non-refusers. The study protocol was approved by the Ethics Committee of the University-Hospital of Kremlin-Bicêtre (France) and written informed consent was obtained from each participant. The participants were free to refuse a specific part of the examination and such refusal did not constitute exclusion criteria, unless they refused the baseline examination. Personal information relating to the participants was kept in secure locations and the participants were identified by a unique number in the dataset.

A total of 2259 participants were included at baseline in the Esprit study. Each participant underwent a half-day examination by a neurologist and a center interviewer (a nurse or psychologist) at the Gui de Chauliac Neurology Hospital (Montpellier, France). The participants who refused or were physically unable to attend the examination center (7% of the participants at baseline) were examined at home.

The participants recruited for the Esprit study underwent structured psychiatric interviews with validated instruments related to depression and anxiety, as well as a standardized interview with questions on socio-demographic, lifestyle characteristics, history of diseases, lifetime stressful events and drugs used (see next chapter for more detail). For women, specific information relating to reproductive history was recorded (age at menarche, age at birth of first and last child, menopause age and type, as well as exogenous hormonal treatment). Participants also reported sleeping habits and insomnia using the Nottingham Health Profile questionnaire (Ribet et al., 1999) and for 340 subjects a night-time physiological measure of sleep was performed at home via polysomnography recording. Blood samples were taken at baseline for the assay of standard biochemical parameters and for the constitution of serum and DNA banks. Another DNA bank has been constituted during the follow-up from buccal mucosa samples. Salivary cortisol was collected in a subgroup of some 400 participants who were not being treated with medication likely to modify cortisol levels (glucocorticoids, hormonal treatment or benzodiazepines) as described previously (Beluche et al., 2009). Subjects were instructed not to drink, eat or smoke for at least 30 mn before saliva collection and to start the protocol at least 1 hour after awakening and then after 3, 7, and 14 hours (the last sample being collected before midnight), recording the exact time. In order to assess the stress reactivity, cortisol secretion was tested in two conditions, one under basal conditions at home and the other under stressful conditions at the hospital.

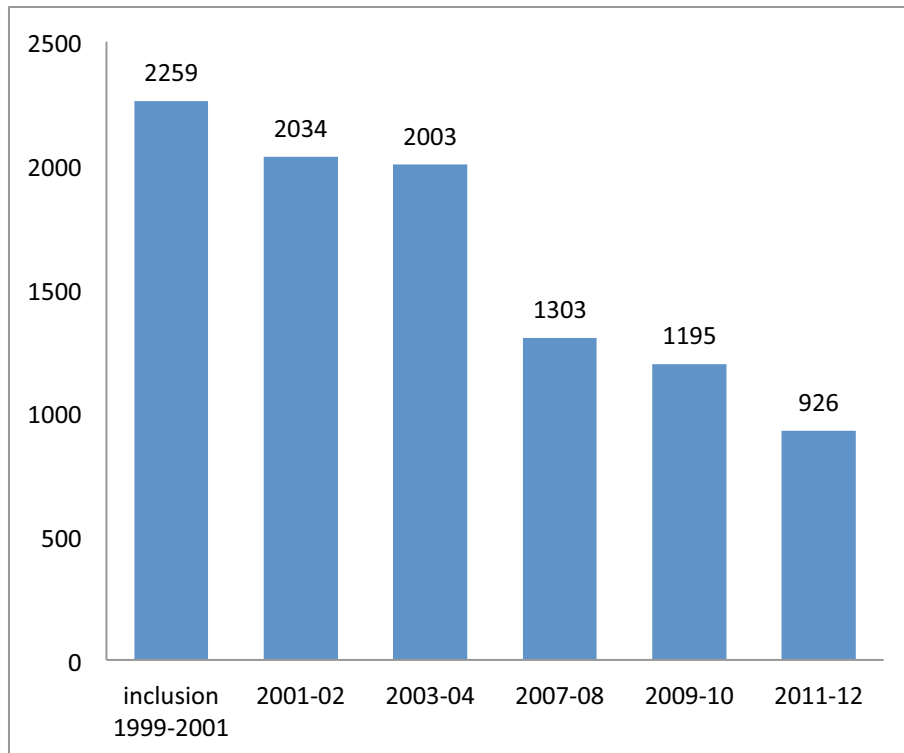
IQ was estimated by the French language adaptation of the National Adult Reading Test

(Mackinnon et al., 1999). The subjects also underwent neurological evaluation as well as vascular clinical examination. Those aged 80 years or less were randomly selected for a brain MRI examination using 1.5 Tesla Magnetom (Siemens, Erlangen, Germany) and data are available on the whole brain, white and grey matter volume, white matter hyperintensities, hippocampus volume, and corpus callosum area (Ryan et al., 2014). Repeated measures of blood pressure in sitting, standing and lying positions were performed using a validated digital electronic tensiometer (Omron Corp. Kyoto, Japan). Participants were also proposed an electrocardiogram (ECG) and carotid ultrasound examinations with scanning of the common carotid artery, the carotid bifurcations, and the origin of the left and right internal carotid arteries and assessment of the presence of plaques.



**Figure 1.** Participant recruitment to the Esprit study.

Participants were re-examined on five further occasions after 2, 4, 7, 10, and 12 years with similar questionnaires as well as medical examinations. The follow-up rate was 89% after 4 years and then declined, reaching 58% after 7 years and 41% after 12 years (**Figure 2**). A 6<sup>th</sup> follow-up wave after 15 years is ongoing.



**Figure 2.** Number of Esprit participants over the 12-year follow-up.

## 1.2 Measures

### 1.2.1 Psychiatric disorder assessment

A standardized psychiatric interview, the Mini-International Neuropsychiatric Interview (French version 5.00), was administered by psychologists and psychiatric nurses to investigate lifetime anxiety disorders (GAD, social phobia, specific phobia and agoraphobia, panic disorder, OCD, and PTSD) and major depression, according to DSM-IV criteria (Ritchie et al., 2013). The MINI is a standardized and structured psychiatric diagnostic examination validated within the general population setting (Lecrubier et al., 1997) which uses a nonhierarchical case identification procedure, thus permitting the diagnosis of psychiatric comorbidities. The interviewers were trained using video recordings of interviews by the clinicians responsible for the development of the French version of the MINI. Following training, the interviewers administered the examination over a 1-month period under the supervision of psychiatrists from the Department of Adult Psychiatry at Montpellier University Hospital. Regular meetings were conducted with clinicians to discuss problems and dual interviews were randomly conducted to minimized interviewer drift. During this training period, false negatives were rare, in agreement with the high negative predictive value of the MINI for GAD (Lecrubier et al.,

1997; Sheehan et al., 1997). In the Esprit study, only positive cases were thus reviewed by a panel of independent psychiatrists to validate the initial diagnosis as described previously (Ritchie et al., 2013). The psychiatrists reviewed cases independently in accordance with DSM-IV criteria, using all information provided by the MINI as recorded by the interviewers, all other available biological and clinical data, and if necessary after consultation with the participant's general practitioner. Consensus agreement was subsequently reached for all cases.

More specifically, current GAD was established using the current definition implying the presence of symptoms for at least six months (American Psychiatric Association, 1994). Part episodes (excluding past 6-months) were also recorded, along with the ages for first and last GAD episode. All the participants were examined at baseline as well as at each follow-up examination. During follow-up, MINI questions referred to the period since the previous examination, two or three years before. For the new cases, the exact date of onset during the follow-up period was not known, and onset was therefore considered to have occurred midway between two examinations. Current phobia includes social phobia and/or agoraphobia in the past month according to DSM-IV criteria.

### **1.2.2. Dementia diagnosis**

Dementia was diagnosed at baseline and each follow-up by a neurologist as part of a standardized clinical examination (The 3C Study Group, 2003). Additional information was also available from neuropsychological evaluation and cognitive tests covering memory, attention, language, and visuo-spatial abilities (see below) as well as data on activities of daily living, MRI or computed tomography scans when available. Finally, an independent committee of neurologists reviewed all suspected dementia cases to obtain a consensus on the diagnosis according to DSM-IV revised criteria as described previously (The 3C Study Group, 2003) and classification into different subtypes; namely probable or possible Alzheimer's disease, vascular dementia, mixed dementia, dementia with Lewy bodies, Parkinson's disease dementia and others types.

### **1.2.3. Socio-demographic, lifestyle, biological and clinical variables**

Of all the data available relating to the participants of the Esprit study, the following specific information was considered further in subsequent analyses.

#### ***Socio-demographic factors***

The standardized interview included questions on socio-demographic characteristics, *e.g.* age, sex, marital status and living situation (married, having a partner, single or divorced, widowed), and education level ( $\geq 5$  years).

#### ***Lifestyle and anthropometric measures***

Information was also available on smoking (current *vs.* ever), alcohol consumption ( $>12$  g/day), physical activity (self-reported frequency of engaging in exercise for fitness or recreational purposes, recorded as regularly /often or not), as well as measures of weight, height, waist (at the midpoint between the lower end of the rib cage and the upper part of the pelvic bone) and hip (at the largest circumference below the waist). Waist-to-hip ratio (WHR) and body mass index (BMI, expressed as  $\text{kg/m}^2$ ) were calculated.

#### ***Physical health***

Information on the health of the participants was obtained through detailed questionnaires, a complete inventory of all medications taken within the preceding month and from venous blood samples taken at baseline after fasting for at least 12 hours. After centrifugation, serum and plasma

(collected in EDTA tubes) samples were stored at -80°C. A number of biochemical parameters were assayed at baseline, *e.g.* glycaemia, creatinine, albumin and prealbumin, fibrinogen, estradiol and testosterone, C- reactive protein and lipids. The total cholesterol, HDL-cholesterol and TG levels were measured in serum using routine enzymatic methods, LDL-cholesterol was determined by the Friedwald formula (Friedewald et al., 1972). A detailed standardized health interview included current and past health status, *e.g.* hypercholesterolemia (defined as a total cholesterol level  $\geq 6.2$  mmol/L or treated by lipid lowering agents or self-reported), hypertension (resting blood pressure  $\geq 160/95$  mmHg or treated), diabetes (fasting glucose level  $\geq 7$  mmol/L or treated), respiratory problems (dyspnea, asthma or bronchitis), osteoporosis and thyroid disorder. Detailed medical questionnaires (with additional information from general practitioners) were used to obtain information on history of ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, and arteritis) as well as arrhythmia and heart failure. We also recorded all somatic and psychotropic medications used in the preceding month, including antidepressant and anxiolytic drugs. Participants were asked to show medical prescriptions, drug packages, and any other relevant information. This enabled more accurate recordings of the exact name and type of medication being used. Medications were identified according to the World health Organisation's ATC classification (WHO, 2000). Disability was measured on a four-point scale ranging, by decreasing level of severity, from confinement to bed, to home, to the neighborhood, to no restriction as described previously (Norton et al., 2012). Visual impairment was defined as having a corrected near visual acuity (Parinaud scale) of more than 4 or difficulties recognizing a familiar face at 4 m, and hearing impairment was defined as deafness or only being able to hear a conversation when a single person speaks loudly (Carriere et al., 2011).

The cognitive tests were administered by trained staff and assessed different areas of cognitive function at baseline and each follow-up. Global cognitive function was measured using the Mini-Mental State Examination (MMSE), which assessed several cognitive functions like orientation in time and space, immediate and delayed recall, attention and calculation, language, and registration. A score  $< 26$  was considered to be indicative of cognitive impairment (Folstein et al., 1975). The Isaacs' Set test (Isaacs et al., 1973) assessed verbal fluency which is sensitive to changes in both frontal and temporal areas. The participants were required to produce as many words as possible within a given semantic category (animals, colors, fruits and cities), and the score was the sum of the number of words generated in each category in 30 seconds. Visual memory was assessed using the Benton Visual Retention test (Benton, 1965) in which participants were presented with a drawing for 10 seconds each consisting of one or more simple geometric designs. The participants were then shown a paper with 4 drawings and were asked to correctly identifying the drawing that they had been just previously shown. Other cognitive tests, the Trail Making Tests (TMT) A and B assessed cognitive skills related to visual scanning, psychomotor speed and executive function (Reitan, 1958). Both TMTA and TMTB consist of 25 circles distributed over a sheet of paper. In TMTA, the circles are numbered 1-25, and the patients are asked to draw lines to connect the numbers in ascending order. In TMTB, the circles include both numbers (1-13) and the letters (A-L), the patients draw lines to connect the circles alternating between the numbers and the letters (1-A-2-B-3-C, etc.). The patients are instructed to connect the circles as quickly as possible, and times to complete A and B tests are recorded. Low cognitive performance was defined as scoring in the lowest tertile except for the timed TMT (highest tertile).

## 1.2.4 Recent and early adverse events

### *Recent adverse life events*

The negative events occurring in the past year were assessed using the Gospel Oak questionnaire (Harwood et al., 1998). This questionnaire is composed 12-item list of major adverse events including bereavement, rupture in significant relationships, financial and legal problems, dismissal, severe illness, and loss of highly valued objects.

## ***Childhood environment***

A retrospective self-report questionnaire (Ritchie et al., 2009) examined the traumatic experiences during childhood and adolescence. It covered 25 adverse and 8 protective factors, which were given to participants for completion at the second follow-up assessment (4 years after recruitment) by which time the interviewers had established close relationships with the subjects, facilitating the request for sensitive information. Participants were asked to respond yes or no to each item. They were also given an opportunity to discuss the questionnaire contents with the interviewer in case of doubt as to whether specific experiences corresponded to the items. Adverse factors examined in the present study included having experienced severe abuse (physical, verbal or sexual abuse, neglect or excessive punishment), parental loss or separation, mental disorder in parents, parents with problems with alcohol or drugs, conflict at home, poverty or financial difficulties and excessive sharing of parental problems as well as war and natural catastrophe. Protective factors included maternal or paternal affection, availability of an adult friend, impression of having had a happy childhood, parents perceived as doing their best, feeling happy at school, having been raised by both parents. Low affective support during childhood was defined as having reported less than six protective factors.

### **1.2.5 DNA extraction and *ADR* genotyping**

DNA extraction was performed either from blood samples taken at baseline or from buccal mucosa samples collected during the follow-up. In both cases, after extraction, DNA samples were stored at -80°C. The DNA samples extracted from white blood cells (Puregene Kit, Qiagen, France) were genotyped with Illumina Human 610-Quad Bead-Chip at the French Centre National de Genotypage in Lille (France). For buccal mucosa samples DNA was extracted and genotyping was performed by LGC Genomics (Hoddesdon Herts, UK) using their competitive allele-specific polymerase chain reaction (PCR) SNP genotyping system (KASPar) as described previously (Ancelin et al., 2013; Freeman et al., 2003). KASP is a competitive allele-specific PCR incorporating a FRET quencher cassette, which has an error rate of less than 0.3%. The amplified PCR products were analyzed by fluorescence scanning in a BMG labtech Pherastar scanner and the results were interpreted with KlusterCaller 1.1 software. For a number of SNPs (including *ADR* variants), genotyping data from both buccal mucosa and blood samples were available for one third of the sample. The results of genotyping from buccal- and blood-cell derived DNA were identical (>99%) despite differences in cell samples, times of collection, extraction methods and genotyping companies, which adds validity to the accuracy of the data.

### **1.2.6 Polymorphisms genotyped in the Esprit study**

In the Esprit study, more than 750 SNPs of at least 250 candidate genes have been selected and genotyped on the basis of their potential involvement in affective disorders and stress. This included variants related to neurogenesis and neurotransmitters (BDNF, COMT, serotonin transporter and receptor,...), autonomous nervous system (ADR, noradrenaline transporter...), HPA axis (glucocorticoid receptor and mineralocorticoid receptor, CRH, CRH receptors, ACTH, Arginine Vasopressin...), steroid and cortisol metabolism (cytochrome P450's, hydroxysteroid dehydrogenases...), sex hormones and hypothalamic-pituitary-gonadal axis (estrogen, androgen, progesterone receptors...). Other candidate genes include inflammatory and circadian genes (capable of mutual regulation and interaction with the stress-HPA axis).

### 1.2.7 Selection of *ADR* polymorphisms

For the genetic study, we used the validated data obtained from buccal sample genotyping. We selected the eight most commonly studied *ADR* polymorphisms that have been associated with physiological stress response related to dysfunction of the adrenergic system in different chronic disorders (Elia et al., 2009; Jin et al., 2008; Liberzon et al., 2014; Lochman et al., 2013). The *ADRA1A* gene is located in chromosomal region 8p21.2 and the chosen SNPs include *rs3808585* in the promoter region and the three intronic SNPs *rs573514*, *rs4732682* and *rs17426222*. The *ADRA2A* gene is located in chromosomal region 10q25.2 and chosen SNPs include *rs1800544* in the promoter region and *rs11195419* located in the 3' untranslated region. The closely positioned *rs7903146* variant is also located in region 10q25.2 in intron 3 of the gene transcription factor 7-like 2 (*TCF7L2*). The *ADRB2* gene is an intronless gene with single exon in chromosomal region 5q31-32. The most extensively studied functional polymorphism is *rs1042713*, a common non-synonymous SNP resulting in an amino acid substitution (otherwise referred to as Arg16Gly). All SNPs except *rs180054* had also been separately genotyped from blood samples at baseline.

## CHAPTER 2 - Statistical analyses

A classical three-step statistical procedure was used involving first a descriptive univariate analysis, then a bivariate analysis and lastly a multivariate analysis. Different statistical models were used in the analyses, depending on the specific research questions being addressed. Logistic regression models were used when examining the factors associated with prevalent GAD and Cox regression models for the predictors of incident GAD. The SAS statistical software (version 9.3, SAS institute, Inc., North Carolina, USA) was used for all statistical analyses and all tests were two-tailed, with the significance level at  $p < 0.05$ , unless otherwise specified.

### 2.1 Statistical methods

Three separate research questions (with 3 corresponding studies) were addressed in this thesis: study A on the prevalence of GAD and its clinical characteristics; study B on the predictors of incident GAD; study C on the role of *ADR* gene polymorphisms in GAD. Studies A and C were performed using a cross-sectional approach and study B was performed using a longitudinal approach.

For each study, only participants with complete data for the variables of interest and free of dementia at baseline, were included in the analyses. For study B, subjects with baseline GAD were also excluded. Subjects excluded and included in each study analysis were compared, using the *t*-test for continuous variables (age) and the two-tailed chi-squared test for categorical variables.

#### 2.1.1 Cross-sectional analysis

##### *Descriptive statistics*

In study A, the basic characteristics of the study population were described, including socio-demographic characteristics, lifestyle as well as physical and mental health. The categorical variables (including prevalent GAD) were expressed as percentages and quantitative variables were summarized using means and standard deviations if they were normally distributed and otherwise medians and interquartile ranges. Normality was tested using the Kolmogorov-Smirnov test.

In study C, the same descriptive statistics as in study A were used to describe the sample given. In addition, the chi-squared test was employed to compare the distribution of *ADR* genotypes with those expected under the Hardy-Weinberg equilibrium.

##### *Bivariate analysis*

This step was performed not only to examine the relationship between the outcome and exposure variables but also to select the covariables. Bivariate associations were examined using minimally adjusted (for age and sex) models constructed for each exposure. Age was entered as a continuous variable after testing the linear relationship with GAD. A fairly low significance level is usually chosen for the selection of covariables to be entered in the subsequent analyses so that potentially important variables will not be missed. Given the large number of covariables, we chose to be conservative and thus selected any variables that were associated with the outcome in the bivariate analysis at the 15% significance level ( $p\text{-value} < 0.15$ ) rather than  $p < 0.25$  as recommended (Hosmer Jr et al., 2000).

In study A, binary analyses adjusted for age and sex were performed to compare the characteristics of participants with and without GAD at baseline, and expressed with odd ratios (OR), 95% confidence intervals (CI), and *p* values. The covariables associated with GAD ( $p < 0.15$ ) were

selected for the next analysis.

In study C, binary analyses adjusted for age and sex were carried out to explore the characteristics of participants with current GAD and the associations between current GAD and *ADR* polymorphisms. Covariates associated with GAD and which could be potential mediating factors (*e.g.* BMI and major depression...) were chosen for the further analyses. This analysis also assessed the associations between *ADR* variants and pure phobia (after the exclusion of the participants with prevalent GAD).

#### *Multivariate analysis*

Multivariate analyses were performed to examine the association between the outcome and a large number of covariates. The result is the independent or adjusted association between the factors of interest and the outcome. In some cases, interaction terms between the covariates were also considered in the models.

In study A, the multivariate analysis included covariates associated with current GAD in the bivariate analysis. Model 1 was first adjusted for socio-demographic, clinical and cognitive variables, and model 2 corresponded to model 1 further adjusted for psychiatric variables. In study C, a similar approach was used for the genetic variables.

### **2.1.2 Longitudinal analysis**

#### *Descriptive statistics*

First, the study population was described at baseline for the main socio-demographic and clinical characteristics. The quantitative variable (age) was summarized using the mean and standard deviation and categorical variables were summarized using numbers and percentages. The incident rate over the 12-year follow-up was calculated for the participants without prevalent GAD at baseline and with data available for at least one of the five follow-up examinations. For the calculation of the incidence rate a participant is counted only once as a case, irrespectively of the number of successive episodes (events) he/she may have experienced during the follow-up, and the date of onset corresponded to the first episode. For the new cases, the exact date of onset during the follow-up period was not known, and onset was therefore considered to have occurred midway between the two examinations. Population incidence was estimated by dividing the number of new cases that occurred during the follow-up by the total number of GAD-free years lived by the cohort from baseline, expressed as number of new cases per 1,000 person-years.

#### *Bivariate analysis*

In study B, longitudinal associations between baseline characteristics and incident GAD (incident GAD: no/yes) were examined using Cox models adjusted for sex. Age was not entered as a covariable since it was already included as the time scale. The proportional-hazards assumptions were tested using Martingale residuals. The baseline characteristics meeting Martingale residual criteria for proportionality of risk and associated with incident GAD in bivariate Cox models ( $p < 0.15$ ) were entered in the final model using a backward selection procedure.

#### *Multivariate analysis*

Baseline covariates meeting Martingale residual criteria for proportionality of risk and associated with incident GAD (GAD, first-onset GAD, or recurrent GAD) were entered into a Cox

model adjusted for sex (model 1). Model 2 was further adjusted for past GAD.

## 2.2 Statistical Models

### 2.2.1 Logistic regression model

Logistic regression is a statistical method (Hosmer Jr et al., 2000) designed to measure the relationship between a binary dependent variable (Y) and one or more variables ( $X_i$ ) which can be qualitative or quantitative. Y characterizes the disease (outcome, GAD) and  $X_i$  the  $i$  factors potentially associated with the disease. This model estimates the odds or likelihood that a given individual will have the outcome or not. Logistic regression models were constructed to investigate, in study A, the socio-demographic, environmental, biological and clinical factors ( $X_i$ ) specifically associated with GAD in the elderly, and in study C, the genetic factors ( $X_i$ ).

The multiple logistic regression model is given by the equation:

$$\text{logit}(\pi) = \log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 x_1 + \dots + \beta_i x_i$$

Where  $\pi$  is a vector with probabilities of “success” for each category,  $\beta_0$  is the intercept and  $\beta_1$ - $\beta_i$  are the estimated regression coefficients associated with  $X_i$ , and  $x_1$ - $x_i$  are each of the particular values of the exposure variables  $X_1$ -  $X_i$ . This model uses a logit link transformation,  $\log\left(\frac{\pi}{1-\pi}\right)$ , which is linearly associated with the variables and is equivalent to the (log) odds of an individual having a particular outcome, for a given set of variables. Thus, each regression coefficient corresponds to the estimated change in the (log) odds of the dependent variable for each one unit change in the independent variable, when all other independent variables are held constant. The regression coefficients are estimated using maximum likelihood estimation, which enables finding the parameter values that maximize the probability of the observed data.

### 2.2.2 Polytomous logistic regression model

The polytomous logistic regression model is a generalization of the binary logistic regression model in which the dependent variable (Y) is polytomous, taking more than two categories. It measures the multinomial response variable Y depending on a set of  $i$  explanatory variables ( $X_1$ -  $X_i$ ). For each outcome category, different regression coefficients were estimated for each variable. The interpretation of the regression coefficients is similar to that for dichotomous logistic regression, and the (log) odds represent the odds or likelihood of the outcome for a specific variable. This model was used to compare the subjects with pure GAD, GAD comorbid with phobia, and pure phobia, with those who were free of GAD and phobia.

### 2.2.3 Cox proportional hazards regression model

The Cox regression model is a statistical method for analyzing time to event data and can be used to evaluate whether an outcome has occurred or not but also the timing of this event over the follow-up (Hosmer et al., 1999). This model provides an estimate of the hazard of an event or survival according to a set of predictor variables. It was used to examine the association between baseline variables and incident GAD over the 12-year follow-up.

The Cox proportional hazards model is given by the equation:

$$h(t, X) = h_0(t) \exp(\beta_1 X_1 + \beta_2 X_2 + \dots + \beta_i X_i)$$

Where time  $t$  is the time variable of choice, *e.g.* age, time since randomization, or time since operation. The  $h(t, X)$  is the hazard at time  $t$ ,  $h_0(t)$  is the baseline hazard at time  $t$  and represents the hazard for an individual with the value 0 for all the predictor variables. The  $\beta_1 \dots \beta_i$  are regression parameters, and  $X_1 \dots X_i$  are a collection of predictor variables. The Cox regression model is semi-parametric and the baseline hazard function is not in general estimated. Each regression coefficient corresponds to the estimated change in the log hazard ratio for a one unit change in the independent variable, when all other independent variables are held constant. In the Cox model, the positive regression coefficient for an explanatory variable means that the hazard of event is high and a negative regression coefficient implies a protective effect to the variables with which it is associated.

Maximum likelihood estimates of the regression coefficients in the Cox models were obtained by a partial likelihood technique. The partial likelihood technique divides the data into risk sets, which is the group of participants at risk for failure at time  $t$ . Participants contribute to the risk set for an event as long as they are under observation at the time the event occurs and share the same baseline hazards function. The partial likelihood then compares participants failing versus those not failing at each time, and the likelihood of an event is modeled. When the participants do not have the event of interest during the study, their last observed follow-up time is less than their potential time to event of interest in the study and they are considered to be right censored.

In the analyses, we aimed to identify potential risk factors for incident GAD over 12 years of follow-up. Age at baseline was used as the basic time scale in the Cox model to help account for the non-proportionality in the risk of incident disease with age among the elderly (Commenges et al., 1998), and the hazard function can be interpreted as the age-specific incidence of GAD. The data involved in the analysis were therefore left-truncated. Cox model with delayed entry was used to explore the risk factors and the participants entered the monitoring period at different ages depending on their age at baseline recruitment.

## **SECTION 4 - RESULTS**

In this section, the first chapter gives the overall design for each of the 3 analyses reported in the thesis (general flowchart) and provides a descriptive analysis of the whole Esprit study population. The diagnosis of lifetime psychiatric disorders (including GAD) was performed by lay interviewers using the MINI (French version 5.00) according to DSM-IV criteria (see Section 3, Chapter 1). The variables and the statistical methods used are detailed in Section 3, Chapters 1 and 2; other specific points are indicated in each article (see below). A brief description of the context and rationale having guided this work and the main aims for each analysis are described below in Chapter 1. The results are then reported in the form of articles addressing the specific aims of this thesis; the first two are published and the third one has been submitted for publication (Chapter 2). The first article concerns the current and lifetime prevalence as well as clinical characteristics associated with GAD in the elderly. The second article evaluates the 12-year incidence rate and characterizes the predictors of GAD in the elderly. The third article examines the association between GAD and the key *ADR* genetic variants in adrenergic-related pathways in the elderly, as well as determines whether adverse lifetime environment could modulate the association (Gene x Environment interactions). A more general discussion of the overall findings of the three articles is given in Section 5 (Chapters 1 to 5), which also includes the strengths and limitations of all the analyses (see Section 5, Chapter 6) and potential implications as well as future perspectives of this work are described in Section 6.

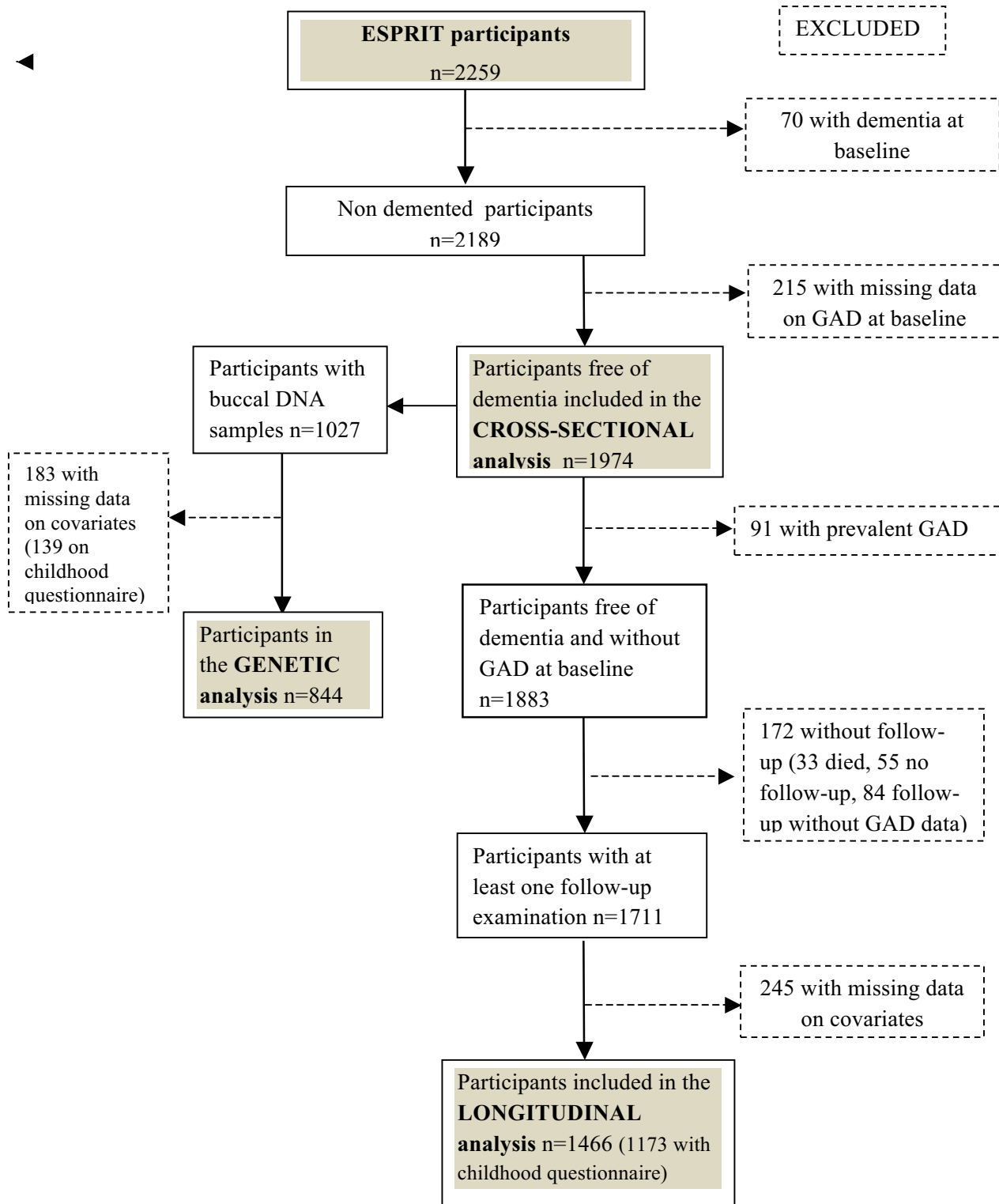
## CHAPTER 1 - Study design and sample

### 1.1 Description of the overall design and general flowchart

A total of 2259 participants aged 65 or over were included in the Esprit study. Of them, 70 with prevalent dementia and 215 with missing data on GAD at the inclusion were excluded from the analyses leaving 1974 subjects (**Figure 3**) for the cross-sectional analyses on prevalent GAD (Section 4, § 2.1).

For the prospective study (Section 4, § 2.2), 91 participants with prevalent GAD at baseline, and 172 participants with missing data on GAD for all follow-up examinations (33 died, 55 were lost to follow-up and 84 had no GAD data) were further excluded from the analysis. The population incidence rate was thus evaluated on 1711 participants with data available for at least one of the five follow-ups. A further 245 subjects with missing data on covariates were excluded from the multivariate analyses leaving 1466 subjects in the final sample.

For the genetic study (Section 4, § 2.3), nearly half of non-demented participants with GAD data at baseline provided buccal mucosa samples for genotyping ( $n=1027$ ). Of them, 844 participants having also completed the childhood questionnaire were considered for the genetic study to examine Gene x Environment interactions.



**Figure 3.** General flow chart of the participants included in the analyses described in the thesis.

## 1.2 Description of the Esprit cohort at baseline

The baseline characteristics of 2259 participants recruited for the Esprit study are given in **Table 6**. Their median age (IQR) was 72 (8) years and 58.1% were females. Forty-five percent had at least 5 years of formal education and 72.5% were either married or living with a partner. Few participants were current smokers (6.5%). More than one third (36.7%) reported physical activity, 46.1% were overweight ( $\text{BMI} \geq 25\text{kg/m}^2$ ), and 22.4% had a WHR higher than 0.94. Nearly two thirds (64.9%) had at least one chronic disorder (mainly hypercholesterolemia and hypertension), 15.7% had ischemic pathologies and 48.8% were taking 4 or more somatic medications. Approximately a quarter of the participants (24.1%) suffered from insomnia, 18.8% had global cognitive impairment, and 3.1% had prevalent dementia. Sixteen percent had current psychiatric disorder, mostly phobia (11.0%), GAD (4.7%) and 15.2% participants were currently taking psychotropic medications. Very few people had PTSD, OCD or panic disorder (<1% for each disorder). Forty percent reported past psychiatric disorders (mainly major depression, phobia, and GAD).

The socio-demographic, lifestyle, biological, and clinical characteristics of the 285 participants excluded from the subsequent analyses differed in some points from that of the 1974 participants included in the cross-sectional analysis. They were older ( $t=10.3$ ,  $p<0.001$ ), with lower education level ( $\chi^2=19.1$ ,  $df=1$ ,  $p<0.0001$ ), less physical activity ( $\chi^2=32.3$ ,  $df=1$ ,  $p<0.0001$ ), lower mobility ( $\chi^2=134.7$ ,  $df=1$ ,  $p<0.0001$ ), more chronic disorders ( $\chi^2=21.5$ ,  $df=1$ ,  $p<0.0001$ ), and cognitive impairment ( $\chi^2=159$ ,  $df=1$ ,  $p<0.0001$ ) and took more somatic medications ( $\chi^2=21.8$ ,  $df=1$ ,  $p<0.0001$ ) as well as psychotropic medication ( $\chi^2=12.1$ ,  $df=1$ ,  $p=0.0005$ ). The representativeness of the subsamples retained for the other analyses are indicated in each article.

**Table 6** Baseline characteristics of the 2259<sup>a</sup> participants in the Esprit study

Variable	N	%
<b>Socio-demographic</b>		
Age [median (IQR)]:	72 (8) years	
Sex (female)	1313	58.1
At least 5 years of education	1024	45.4
Married or living with a partner	1635	72.5
<b>Lifestyle</b>		
Smoking:		
current	147	6.5
past	801	35.5
High alcohol consumption (>12g/day)	857	38.9
Physical activity	734	36.7
Body mass index ( $\geq 25$ kg/m <sup>2</sup> )	1033	46.1
Waist-to-hip ratio (WHR $\geq 0.94$ )	452	22.4
Recent adverse events	1278	59.4
<b>Physical health</b>		
At least one chronic disorder <sup>b</sup>	1467	64.9
Number of somatic medications ( $\geq 4$ )	1103	48.8
Hypercholesterolemia (cholesterol $\geq 6.2$ mmol/L or treated)	1247	55.9
Hypertension (resting blood pressure $\geq 160/95$ mm Hg or treated)	1033	45.7
Diabetes (glycemia $\geq 7$ mmol/L or treated)	218	9.8
Ischemic pathologies <sup>d</sup>	354	15.7
Arrhythmia and heart failure	328	14.6
Respiratory disorders (dyspnea, asthma, or bronchitis)	137	6.1
Insomnia	525	24.1

Variable	N	Percent %
Cognitive score at baseline (MMSE<26)	421	18.8
Mobility limitation <sup>c</sup>	171	7.6
<b>Mental health</b>		
Current psychiatric disorder	325	16.4
Past psychiatric disorder	799	40.0
Current major depression	59	3.0
Past major depression	501	25.0
Current GAD	94	4.7
Past GAD	127	6.3
Current phobia	221	11.0
Past phobia	390	19.4
Current post-traumatic stress disorder	5	0.3
Past post-traumatic stress disorder	14	0.7
Current panic disorder	6	0.3
Past panic disorder	32	1.6
Current obsessive compulsive disorder	12	0.6
Past obsessive compulsive disorder	21	1.0
Psychotropic medication	343	15.2
Dementia	70	3.1

<sup>a</sup> The total number of subjects for each characteristic may be less than the sample size because of missing data which never exceeded 4% except for physical activity (11%), WHR (11%), and certain psychiatric disorders ( $\leq$  11% missing data).

<sup>b</sup> Chronic disorders correspond to hypercholesterolemia, hypertension, diabetes, asthma, osteoporosis, thyroid disorder, and recent cancer.

<sup>c</sup> Mobility limitation corresponds to confinement to bed, to the home or to one's neighborhood.

<sup>d</sup> Ischemic pathologies correspond to angina pectoris, myocardial infarction, stroke, cardiovascular surgery, and arteritis.

### **1.3. Rationale and objectives of the work**

#### **1.3.1 GAD prevalence and clinical characteristics in elderly general population (Article 1)**

Anxiety disorders are the most prevalent psychiatric disorders affecting about one in four adults across the lifetime and over 10% of individuals in late-life (Kessler et al., 2007a). GAD is one of the most common anxiety disorders in older adults and is associated with increased disability (Norton et al., 2012) and premature mortality (Carrière et al., 2013). However, GAD is frequently ignored and undiagnosed because general practitioners and patients tend to pay more attention to physical symptoms. In addition, it is generally assumed that high rates of anxiety are to be expected in elderly persons due to increased vulnerability with ageing (Parmentier et al., 2013). In fact, GAD is generally assumed to be the continuing chronic course of an early onset of illness in the elderly. Besides, most studies in the elderly are carried out in clinical settings rather than in community-dwelling elderly populations, with a lack of information on lifetime prevalence rates, treatment, and overall clinical characteristics, which may be specific to older persons.

The purpose of the first study is to estimate the current and lifetime prevalence rates of late-life GAD in a large cohort of French community-dwelling elderly people (the Esprit study). We aim also to describe the main characteristics of the disease (age of onset, chronicity, and treatment) as well as the factors specifically associated with GAD in the elderly, notably focusing on comorbidity with other psychiatric and physical disorders (Section 4, § 2.1).

#### **1.3.2 Risk factors for incident GAD in the elderly (Article 2)**

The incidence rates and predictors of GAD have rarely been studied in elderly general populations and the predictors of late-onset GAD are generally assumed to be the same as for early onset GAD. Only two prospective studies have been performed in elderly people, both limited by only one follow-up examination over 3 years and thus a lower number of cases and statistical power. Very few candidate risk factors (mainly psychopathological) were identified (Chou et al., 2011; Schoevers et al., 2005). Other predictors, including biological and clinical characteristics and lifetime adverse environment, have not been thoroughly investigated although the identification of risk factors for GAD onset could be essential for prevention as well as clinical management and intervention.

This second study is aimed at estimating the incidence rate of GAD in this elderly population over 12-years of follow-up and characterizing the predictors of incident GAD in late life among a large range of socio-demographic, behavioral, environmental (early and late lifetime adverse events), biological, and clinical risk factors (Section 4, § 2.2).

#### **1.3.2 Adrenergic receptor gene variants and GAD (Article 3, submitted)**

Genetic risk factors for GAD have rarely been examined despite a relatively high heritability, previous family studies suggesting a substantial genetic contribution to GAD of up to 30% (Domschke et al., 2012; Sartor et al., 2012). However, few candidate genes have been identified, and none in the elderly. The rare studies have examined principally neurotransmitter-related genes, and have reported relatively small effects and possible associations with a number of other psychiatric disorders too (Tadic et al., 2003; You et al., 2005). A targeted strategy driven by pathobiochemical mechanisms could be better suited for hypothesis-testing regarding the specific involvement of genes susceptible to play a key role in the disease etiology. This requires the identification of the main physiopathological mechanisms whose dysfunction could be associated with vulnerability to late-life GAD.

The third study examines specific genetic risk factors for GAD in this elderly population and potential modulations or interactions with adverse environmental factors. The choice of the *ADRA* variants was based on the findings of two previous studies which showed very specific late-life characteristics of GAD in the elderly not shared with other major psychiatric disorders in the elderly and which also supported a critical role of stress systems, especially the adrenergic nervous system in GAD in particular in response to stressful adverse life events (Section 4, § 2.3).

## **CHAPTER 2 - Articles**

### **2.1 Generalized anxiety in community-dwelling elderly: prevalence and clinical characteristics**



## Brief report

## Generalized anxiety in community-dwelling elderly: Prevalence and clinical characteristics



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## ABSTRACT

**Background:** Generalized anxiety disorder (GAD) is a chronic and disabling disorder with a low rate of full remission. As it is commonly assumed that cases in the elderly principally represent the continuing chronic course of early onset illness, there has been little research into the clinical characteristics, including comorbid psychiatric and physical conditions, which may be specific to older people.

**Methods:** Lifetime GAD and psychiatric comorbidity were diagnosed in 1974 community-dwelling elderly people aged 65 or over using a standardized psychiatric examination, the MINI, based on DSM-IV criteria. Multivariate regression analyses were adjusted for socio-demographic, lifestyle, biological, and clinical variables, as well as adverse life events.

**Results:** The lifetime prevalence of GAD was 11% (95% CI=9.6–12.4%) of whom 24.6% reported a late onset with a first episode after 50 years of age. The 6-month current prevalence was 4.6% (95% CI=3.7–5.5%). Most of the prevalent cases were recurrent but only 36.3% were receiving treatment. Fourteen percent were comorbid with major depression and 34% with phobia but their associated factors differed. The factors associated with pure GAD were being female, having cognitive impairment, lower body mass index, reporting low affective support during childhood, taking a high number of somatic medications independently of other mental health factors, e.g. psychotropic medication use, major depression, and phobia.

**Limitations:** The study is limited by cross-sectional design.

**Conclusions:** Our data indicate that GAD prevalence is high in elderly people with a late-life onset of GAD in 25% of cases. GAD in the elderly is not just a severity marker of depression and is clinically distinct from phobia, the other major anxiety disorder of the elderly.

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## 1. Introduction

Generalized anxiety disorder (GAD) is a chronic disorder commonly preceding depressive episodes and associated with increased disability and mortality (Kessler et al., 2001). Treatment is difficult with low rates of full remission (Hoge et al., 2012). Despite a high prevalence in primary care, its recognition in general practice is relatively low, especially in older adults (Parmentier et al., 2013). It is indeed commonly assumed that cases in the elderly represent the continuing chronic course of early onset illness and/or a severity marker of depression (Kessler

and Wittchen, 2002; American Psychiatric Association, 1994). However, different risk profiles may be expected among the elderly in comparison with younger adults as both the exposure to and the impact of risk factors change with age (Vink et al., 2008). This notably includes lifetime accumulation of traumatic events as well as chronic physical and neuropsychiatric disorders (cognitive decline, depression, and other anxiety disorders which are frequent in the elderly (Ritchie et al., 2004), especially phobia, which also have specific characteristics (Ritchie et al., 2013)).

Previous studies have been mostly carried out in clinical settings, which limits generalizability; community-based studies have tended to use symptom scales as opposed to structured clinical interviews and rarely examine older adults specifically (Vink et al., 2008). Four principal epidemiological studies focusing on GAD in elderly populations mainly found associations with the number of chronic disorders, functional limitations, and psychosocial factors (Beekman

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et al., 2000; Schoevers et al., 2003, 2005; Goncalves et al., 2011; Chou et al., 2011). None of them considered psychotropic use as well as factors associated with early environment, or related to specific age-related chronic disorders.

This study aimed to describe lifetime GAD prevalence for both early and late-life onset cases and their clinical characteristics including comorbidity in a large cohort of over 2000 community-dwelling elderly. Psychiatric disorder was detected using a standardized clinical interview and controlling for a large range of socio-demographic, lifestyle, and clinical variables as well as early and late-life adverse events.

## 2. Methods

### 2.1. Subjects

Participants  $\geq 65$  years old were recruited by random selection from electoral rolls between 1999 and 2001 as part of the ESPRIT study of neuropsychiatric disorders in community-dwelling French elderly people (Ritchie et al., 2004). Of the persons initially contacted, 27.3% refused to participate and were replaced by another participant drawn randomly from the same electoral division so that each division was equally represented. The protocol was approved by the National Ethics Committee and written informed consent was obtained. Of the 2189 non-demented participants, 215 were excluded because of missing data on GAD at baseline, leaving 1974 subjects for the analyses. Their socio-demographic, lifestyle, biological and clinical characteristics were not significantly different from those excluded.

### 2.2. Clinical measures and socio-demographic, lifestyle, biological characteristics

The diagnosis of lifetime anxiety disorder and major depression was established according to DSM-IV criteria using the Mini-International Neuropsychiatric Interview (MINI, French version 5.00), a standardized psychiatric interview validated within the general population setting (Lecrubier et al., 1997). Interviewers were trained for 3 months in the Department of Adult Psychiatry at La Colombière Hospital (Montpellier, France), and cases were reviewed by a panel of independent psychiatrists as described previously (Ritchie et al., 2013).

A standardized interview included questions on socio-demographic characteristics, smoking, alcohol, physical activity, diabetes, respiratory disorders, osteoporosis, thyroid disorder, cancer, hypercholesterolemia, hypertension, and measures of weight, height, waist, and hip. Waist-to-hip ratio (WHR) and body mass index (BMI, expressed as  $\text{kg}/\text{m}^2$ ) were calculated. Medical questionnaires provided information on history of ischemic pathologies (angina, myocardial infarction, stroke, cardiovascular surgery, and arteritis) as well as arrhythmia and heart failure. The participants were asked to show medical prescriptions, drug packages, and any other relevant information to record all past-month somatic and psychotropic medications taken. Mobility limitation, visual and hearing impairment were evaluated as described elsewhere (Norton et al., 2012). Lipid levels were measured from blood samples taken after 12 h-fasting (Ancelin et al., 2010), and global cognitive function using the Mini-Mental State Examination (MMSE), a score  $< 26$  indicating cognitive impairment (Folstein et al., 1975). Verbal fluency and visual memory were assessed using Isaacs' Set (Isaacs and Kennie, 1973) and the Benton Visual Retention Test (Benton, 1965). The Trail Making Tests (TMT) A and B assessed psychomotor speed and executive function (Reitan, 1958). Low cognitive performance was defined as scoring in the lowest tertile except for the timed TMT

(highest). Exposure to adverse events in the past year was assessed using the Gospel Oak questionnaire (Harwood et al., 1998). A self-report questionnaire (Ritchie et al., 2009) with binary yes/no response categories examined environment during childhood and adolescence, covering exposure to severe abuse (physical, verbal or sexual abuse, neglect or excessive punishment), parental loss or separation, parents with mental disorder, alcohol or drugs problems, conflict at home, financial difficulties, excessive sharing of problems, war and natural catastrophe. Low affective support was defined as having reported less than six positive factors among parental affection, availability of an adult friend, happy childhood, happy at school, normal education, parents doing their best, and raised by both parents.

### 2.3. Statistical analysis

Chi-square tests compared the characteristics of participants included in the analyses with those excluded. Logistic regression was used to compare participants with or without GAD at baseline and polytomous regression to compare subjects with pure GAD, GAD comorbid with phobia, and pure phobia, with subjects free of GAD and phobia. Multivariate models included covariates associated with GAD ( $p < 0.15$ ). Model 1 was adjusted for socio-demographic and clinical variables and Model 2 was further adjusted for psychiatric variables (including use of psychotropic medication, current major depression and phobia). SAS (version 9.3, SAS Institute, NC, USA) was used for the statistical analysis and all tests were two-tailed, with the significance level at  $p < 0.05$ .

## 3. Results

### 3.1. Socio-demographic and clinical characteristics associated with current GAD

In this elderly sample (58.3% females), the 6-month prevalence of GAD was 4.6% (95% CI = 3.7–5.5%). The mean(SD) age of the sample was 72.8(5.3) years with no significant differences between subjects with and without GAD. Current GAD was 2.2-fold more frequent in women (6.0% vs. 2.7%,  $p = 0.0007$ ). Around 36.3% of the GAD cases were taking psychotropic medication (compared to 13.1% without GAD,  $p < 0.0001$ ); 42.5% anxiolytics, 33.3% antidepressants and 24.2% both. In analyses adjusted for age and sex, a higher education level, higher BMI, and lower HDL-cholesterol were significantly associated with a lower odds of GAD (Table 1). GAD was associated with lower performance on the Isaacs task, hearing impairment, number of somatic medications, depression, phobia, psychotropic medication, and recent adverse events. Multivariate logistic regression (Model 1) showed a higher number of somatic medications and female sex being associated with GAD and higher BMI with lower prevalence (Supplementary Table S1). After further adjustment for psychiatric disorder (Model 2), the association with higher BMI was significant (OR = 0.59, 95% CI = 0.35–0.98,  $p = 0.04$ ) as well as that with psychotropic medication (OR = 2.62, 95% CI = 1.56–4.40,  $p = 0.0003$ ), depression (OR = 2.80, 95% CI = 1.29–6.11,  $p = 0.01$ ), and phobia (OR = 3.72, 95% CI = 2.22–6.23,  $p < 0.0001$ ). The impact of early environment was examined in 1352 participants having completed the childhood questionnaire. In the fully adjusted model (Model 2), low affective support was independently associated with GAD (OR = 1.98, 95% CI = 1.00–3.91,  $p = 0.05$ ).

### 3.2. Psychiatric comorbidity

Fourteen percent of GAD cases were comorbid with major depression, and 38.2% with other anxiety disorders, which was predominantly phobia of all types (for 34.8%) (cf. Table 1). Less

**Table 1**  
 Characteristics of participants according to prevalent GAD in an elderly cohort (n=1974<sup>a</sup>).

Characteristic	No GAD (N=1883)		GAD (N=91)		GAD vs. no GAD		
	N	%	N	%	OR <sup>b</sup>	95% CI	p
<b>Socio-demographic</b>							
Living alone	503	26.76	27	29.67	0.88	0.53–1.45	0.60
Childless	183	10.26	6	7.06	0.61	0.26–1.43	0.26
Education level ( $\geq 5$ years)	898	47.74	31	34.07	0.62	0.39–0.96	0.03
<b>Lifestyle</b>							
Alcohol consumption ( $> 12$ g/day)	735	39.75	26	29.21	0.86	0.51–1.44	0.56
Smoking (current or ever)	797	42.35	36	39.56	1.36	0.84–2.19	0.21
Physical activity	651	39.29	28	34.15	0.81	0.50–1.30	0.37
Body mass index (BMI $\geq 25$ kg/m <sup>2</sup> )	875	46.87	29	32.22	0.61	0.38–0.96	0.03
Waist-to-hip ratio (WHR $\geq 0.94$ )	385	22.33	11	13.10	0.79	0.37–1.67	0.54
<b>Lifetime adverse events</b>							
Recent adverse events <sup>c</sup>	1071	58.37	60	68.18	1.48	0.93–2.35	0.10
Childhood events:							
Severe abuse	180	12.86	14	23.33	2.03	1.09–3.78	0.03
Parental loss or separation	479	34.21	19	31.67	0.88	0.50–1.53	0.64
Parents with mental problems	269	19.21	17	28.33	1.50	0.83–2.68	0.18
Parents had problems with alcohol or drugs	106	7.57	10	16.67	2.32	1.14–4.74	0.02
Conflict, nervous stress at home	228	16.29	11	18.33	1.08	0.55–2.12	0.83
Poverty, financial difficulties	323	23.07	18	30.00	1.55	0.88–2.75	0.13
Parents too often shared their problems with children	188	13.43	12	20.00	1.53	0.79–2.94	0.21
Parent or adult friend affection	1140	81.43	42	70.00	0.55	0.31–0.97	0.04
Low affective support	691	36.70	49	53.85	2.03	1.32–3.10	0.001
War or natural catastrophe	763	54.50	36	60.00	1.26	0.74–2.15	0.40
<b>Biological and clinical variables</b>							
LDL-cholesterol ( $> 4.01$ mmol/l)	599	32.31	30	33.33	0.99	0.63–1.55	0.96
HDL-cholesterol ( $< 1.73$ mmol/l)	1245	66.79	46	51.11	0.63	0.41–0.99	0.04
TG ( $\geq 0.95$ mmol/l)	1250	67.06	52	57.78	0.72	0.47–1.12	0.13
Hypercholesterolemia (cholesterol $\geq 6.2$ mmol/l or treated)	1040	55.61	55	60.44	1.08	0.70–1.67	0.74
Hypertension (resting blood pressure $\geq 160/95$ mmHg or treated)	844	44.82	37	40.66	0.89	0.58–1.38	0.60
Diabetes (glycemia $\geq 7$ mmol/l or treated)	168	9.01	6	6.59	0.84	0.36–1.97	0.69
Ischemic pathologies <sup>d</sup>	272	14.45	11	12.09	0.99	0.51–1.91	0.97
Arrhythmia and heart failure	250	13.33	16	17.58	1.41	0.80–2.48	0.23
Respiratory disorders (dyspnea, asthma, or bronchitis)	106	5.63	6	6.59	1.27	0.54–3.00	0.58
Osteoporosis	343	18.42	19	20.88	0.82	0.48–1.41	0.48
Thyroid disorder	148	7.92	8	8.79	0.90	0.42–1.91	0.78
At least one chronic disorder <sup>e</sup>	1190	63.20	57	62.64	1.04	0.67–1.62	0.87
MMSE ( $< 26$ )	273	14.58	20	21.98	1.54	0.92–2.58	0.10
Isaacs Set test score (lowest tertile)	514	27.75	34	37.36	1.67	1.07–2.61	0.02
Benton Visual Retention Test score (lowest tertile)	406	21.75	25	27.47	1.35	0.84–2.18	0.22
Trail Making Test A score (highest tertile)	556	29.94	33	36.67	1.32	0.84–2.08	0.22
Trail Making Test B score (highest tertile)	544	30.26	35	38.89	1.46	0.94–2.28	0.10
Visual impairment	117	6.66	6	7.41	1.06	0.45–2.52	0.89
Hearing impairment	75	4.00	8	8.79	2.68	1.24–5.80	0.01
Mobility limitation <sup>f</sup>	94	5.01	7	7.69	1.53	0.66–3.60	0.32
Number of somatic medications $\geq 4$	869	46.15	58	63.74	2.01	1.28–3.14	0.002

Current mental health	247	13.12	33	36.26	3.48	2.20–5.49	<0.0001
Use of psychotropic medication	45	2.41	13	14.44	6.27	3.22–12.22	<0.0001
Major depression	196	10.46	34	38.20	4.78	3.02–7.56	<0.0001
Anxiety disorder (without GAD)	185	9.87	31	34.83	4.39	2.75–7.01	<0.0001
All types of phobia	3	0.16	2	2.20			n.a.
Post-traumatic stress disorder	5	0.27	0	0.00			n.a.
Panic disorder	7	0.37	4	4.40			n.a.
Obsessive compulsive disorder							

n.a.: not applicable due to the low number of participants.

<sup>a</sup> The total number of subjects for each characteristic may be less than the sample size because of missing data which never exceeded 5.3% except for physical activity (12%), WHR (8%), and visual impairment (7%) as well as childhood events whose questionnaire was completed by 1352 subjects.

<sup>b</sup> Adjusted for age (continuous) and sex.

<sup>c</sup> At least one recent adverse event during the past year assessed using the Gospel Oak questionnaire (Harwood et al., 1998).

<sup>d</sup> Ischemic pathologies correspond to angina pectoris, myocardial infarction, stroke, cardiovascular surgery, and arthritis.

<sup>e</sup> Chronic disorders correspond to hypercholesterolemia, hypertension, diabetes, asthma, osteoporosis, thyroid disorder, and recent cancer.

<sup>f</sup> Mobility limitation corresponds to confinement to bed, to the home or to one's neighborhood.

than 5% of the GAD cases were comorbid with another anxiety disorder. Variables associated at  $p < 0.15$  with GAD/phobia comorbidity in a polytomous regression model were entered into a multivariate model. Being female, having a lower Isaacs score, depression, taking a high number of somatic medications and psychotropic medication were significantly associated with GAD without phobia whereas higher BMI was associated with lower odds (Table 2). Some associations were also found for current phobia without GAD (sex, Isaacs score, and depression) but none were found with BMI and psychotropic medication despite a 3-fold higher number of phobia cases. Conversely, younger age and lower education level were associated with phobia specifically. Psychotropic medication and depression were associated with GAD comorbid with phobia.

### 3.3. Characteristics of late onset GAD

The lifetime prevalence of GAD was 11.0% (95% CI=9.6–12.4%), and the median(IQR) age of first onset was 35(28) years. Of the participants with past but not current GAD, 20.6% were taking psychotropic medication (anxiolytics 46.1%, antidepressants 23.1%, and both 30.8%). All participants with current GAD except one reported past GAD episodes, with a late onset (after 50 years of age) for 24.6%. In a logistic regression analysis comparing early and late onset GAD adjusted for age, sex, and education level, lower HDL-cholesterol (OR=0.47, 95% CI=0.22–1.03,  $p=0.06$ ) and thyroid disorder (OR=2.75, 95% CI=0.95–7.91,  $p=0.06$ ) were associated with late-onset GAD along with a worse verbal fluency (OR=2.88, 95% CI=1.36–6.10,  $p=0.006$ ). No significant differences were found regarding the other cognitive tasks ( $p > 0.13$ ), depression ( $p=0.86$ ), phobia ( $p=0.33$ ), and psychotropic medication ( $p=0.84$ ).

## 4. Discussion

In this large community-dwelling elderly sample, we observed a 6-month prevalence of GAD of 4.6% (95% CI=3.7–5.5%) with a female to male ratio of 2.2. The lifetime prevalence was 11.0% (95% CI=9.6–12.4%) with a median age of first onset of 35 years with 24.6% of cases occurring over age 50. The higher prevalence rate compared to some studies (Schoevers et al., 2003; Goncalves et al., 2011; Alonso and Lépine, 2007) could be due to differences in reference periods (1, 6, or 12 months), heterogeneity in sample and age (all age, young adults, or elderly people), diagnostic instruments (MINI, and CIDI), and criteria (ICD-10, DSM-III or -IV). We used a standardized clinical interview based on DSM-IV criteria with clinical validation of cases, giving more accurate case identification than in some previous studies (Beekman et al., 2000; Schoevers et al., 2003).

Despite the use of psychotropic medication, 36.3% of treated cases still met the criteria for GAD and 63.7% received no treatment which suggests under-recognition and/or inappropriate treatment. Nearly all of the prevalent GAD cases were recurrent, 14.4% were comorbid with major depression, and 34.8% with phobia, whereas less than 5% of the GAD cases were comorbid with another anxiety disorder. Adjustment or exclusion of depressed participants did not change our findings. There was some overlap in the factors associated with pure GAD and pure phobia (sex, verbal fluency, and depression), others were specific to pure GAD (BMI and psychotropic medication), whereas age and education level were associated with phobia specifically. GAD comorbid with phobia appeared closer to GAD than phobia. A French study of 36,105 young adults reported, using the MINI, higher rates of prevalent GAD (12.8%) and depression comorbidity (25.6%), but a lower comorbidity with other anxiety disorder (Leray et al., 2011). They found similar factors associated with

**Table 2**Multivariate polytomous regression analysis of current GAD according to comorbidity with all types of phobia<sup>a</sup>.

Characteristic	Global <i>p</i> - Value	Only GAD ( <i>n</i> =59)		GAD comorbid with phobia ( <i>n</i> =30)		Only phobia ( <i>n</i> =179)	
		OR [95% CI] <i>p</i>		OR [95% CI] <i>p</i>		OR [95% CI] <i>p</i>	
Age (continuous)	0.08	0.96 [0.91–1.02]	0.17	0.96 [0.89–1.03]	0.27	0.97 [0.94–1.00]	0.03
Sex (female)	< 0.0001	2.20 [1.15–4.21]	0.02	1.32 [0.58–2.99]	0.51	2.41 [1.67–3.50]	< 0.0001
Education level ( $\geq 5$ years)	0.01	0.65 [0.36–1.16]	0.14	0.56 [0.26–1.23]	0.15	0.62 [0.44–0.87]	0.006
Isaac Set test score (lowest tertile)	0.01	1.86 [1.05–3.30]	0.03	0.66 [0.27–1.63]	0.37	1.54 [1.09–2.19]	0.01
Body mass index (BMI $\geq 25$ kg/m <sup>2</sup> )	0.10	0.56 [0.32–1.00]	0.05	0.51 [0.23–1.16]	0.11	1.02 [0.74–1.41]	0.92
Hearing impairment	0.34	2.46 [0.91–6.63]	0.08	0.78 [0.10–6.25]	0.82	0.95 [0.40–2.27]	0.92
Number of somatic medications $\geq 4$	0.03	1.85 [1.05–3.28]	0.03	1.85 [0.84–4.08]	0.13	1.36 [0.98–1.88]	0.07
Use of psychotropic medication	0.0008	2.58 [1.43–4.67]	0.002	2.81 [1.23–6.42]	0.01	0.82 [0.52–1.31]	0.40
Current major depression	< 0.0001	3.88 [1.53–9.84]	0.004	7.74 [2.74–21.91]	0.0001	3.40 [1.66–6.95]	0.0008

<sup>a</sup> The analysis was performed with the 1893 subjects having no missing data on any covariates. The reference corresponds to the subjects without GAD and without phobia (*n*=1625).

GAD, phobia, and panic disorder, female gender, younger age, low income, depression, and drug addiction. Our data indicate that GAD in older age is not just a severity marker of depression and has specific characteristics, notably distinct from phobia.

We found that a high number of somatic medications was associated with GAD, consistent with reported associations with a high number of chronic illnesses (Schoevers et al., 2003). We found no associations with functional limitations in contrast to some studies (Schoevers et al., 2005; Goncalves et al., 2011; Beekman et al., 1998) which may be due to the low number of disabled people in this sample. We observed an association with a specific area of cognitive impairment, verbal fluency that was not found in previous studies restricted to the MMSE (Schoevers et al., 2005; Beekman et al., 1998). Furthermore, verbal fluency was the only cognitive task associated with late onset. A few small case-control studies suggest an association between GAD and deficits in cognitive control including fluency (Beaudreau et al., 2013). The negative association between BMI and GAD was more surprising although this had also been reported with anxiety symptoms (Rivenes et al., 2009) and GAD (Hasler et al., 2004; Mazzeo et al., 2004). This finding could be explained by reverse causality, those with prevalent GAD having a lower BMI as a result of symptoms and declining health.

We found that low affective support during childhood was independently associated with GAD. Negative parenting behavior and insecure attachment have been associated with GAD in children and young adults (Newman et al., 2013; Beesdo et al., 2010). Our data indicated that such adverse environmental factors remained associated with GAD even more than 50 years later. Stressful events have been associated with marked long-term changes in brain circuitry regulating stress reactivity involving the HPA axis (Faravelli et al., 2012). We have previously reported in this cohort an association between GAD and higher cortisol secretion (Chaudieu et al., 2008) which suggests a mediating role of the HPA axis.

A limitation of this study was its cross-sectional design which limits causal inferences except for childhood environment which however, relied on retrospective recall. We could not exclude bias due to refusal of a disabled group which may have led to an underestimation of the actual case number. The possibility remained that unmeasured factors such as personality traits may also be involved despite extensive adjustments for a large number of confounders, particularly lifestyle, adverse events, physical and mental comorbidity. Other strengths were the study design, i.e. a large sample of community-dwelling elderly people and the differential diagnosis of the anxiety disorders as well as depression using a standardized and validated psychiatric examination based on DSM-IV criteria, thus minimizing misclassification. Medical history, medication use from prescriptions and drug packages, and neuropsychological assessment were also considered.

Our study provides novel information on GAD in the general elderly population, being not simply as an offside-effect of other psychopathology and associated with depression and phobia more frequently than any other anxiety disorder. This study suggests longstanding vulnerability involving early environmental factors as well as proximal ageing-related factors such as cognitive impairment, psychiatric comorbidity, and somatic burden. Clinically, these findings challenge existing assumptions that late-life GAD is the continuity of a disorder of early adulthood, with 25% of cases appearing after age 50. They may also contribute to better identification of the disorder in elderly persons where it is not only frequent but poorly recognized and undertreated.

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#### Conflict of interest

The authors report no competing interests.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jad.2014.09.036>.

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## Supplementary data

**Table S1** Multivariate logistic regression of current GAD (n=1825, 84 events)

Characteristic	Model 1			Model 2		
	OR	95%CI	p	OR	95%CI	p
Age (continuous)	0.97	0.93-1.02	0.20	0.97	0.93-1.02	0.20
Sex (female)	1.70	0.99-2.92	0.05	1.21	0.75-2.30	0.34
Education level ( $\geq 5$ years)	0.65	0.41-1.05	0.08	0.69	0.42-1.14	0.14
Recent trauma	1.46	0.90-2.36	0.12	1.36	0.83-2.23	0.23
Body Mass Index (BMI $\geq 25$ kg/m <sup>2</sup> )	0.54	0.33-0.89	0.02	0.59	0.35-0.98	0.04
HDL-cholesterol (<1.73mmol/l)	0.71	0.44-1.13	0.15	0.66	0.40-1.07	0.09
Hearing impairment	2.06	0.84-5.03	0.11	2.10	0.82-5.36	0.12
Isaacs Set test score(lowest tertile)	1.40	0.86-2.27	0.18	1.17	0.70-1.96	0.54
Number of somatic medications $\geq 4$	1.96	1.23-3.12	0.01	1.61	0.99-2.60	0.06
Use of psychotropic medications				2.62	1.56-4.40	0.0003
Current major depression				2.80	1.29-6.11	0.01
Current phobia (all types)				3.72	2.22-6.23	<0.0001

## **2.2 Risk factors for late-onset generalized anxiety disorder: results from a 12-year prospective cohort (The Esprit study)**

## ORIGINAL ARTICLE

## Risk factors for late-onset generalized anxiety disorder: results from a 12-year prospective cohort (The ESPRIT study)

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Generalized anxiety disorder (GAD) is a chronic and highly prevalent disorder associated with increased disability and mortality in the elderly. Treatment is difficult with low rate of full remission, thus highlighting the need to identify early predictors for prevention in elderly people. The aim of this study is to identify and characterize incident GAD predictors in elderly people. A total of 1711 individuals aged 65 years and above and free of GAD at baseline were randomly recruited from electoral rolls between 1999 and 2001 (the prospective ESPRIT study). The participants were examined at baseline and five times over 12 years. GAD and psychiatric comorbidity were diagnosed with a standardized psychiatric examination, the Mini-International Neuropsychiatry Interview on the basis of DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition) criteria and validated by a clinical panel. During the follow-up, 8.4% (95% confidence interval = 7.1–9.7%) of the participants experienced incident GAD, 80% being first episodes; the incident rate being 10 per 1000 person-years. The principal predictors of late-onset incident GAD over 12 years derived from a multivariate Cox model were being female, recent adverse life events, having chronic physical (respiratory disorders, arrhythmia and heart failure, dyslipidemia, cognitive impairment) and mental (depression, phobia and past GAD) health disorders. Poverty, parental loss or separation and low affective support during childhood, as well as history of mental problems in parents were also significantly and independently associated with incident GAD. GAD appears as a multifactorial stress-related affective disorder resulting from both proximal and distal risk factors, some of them being potentially modifiable by health care intervention.

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## INTRODUCTION

Generalized anxiety disorder (GAD) is a chronic and relatively frequent disorder with lifetime prevalence rates of 5–10%. It commonly precedes major depression and is associated with increased disability, mortality and suicide attempts.<sup>1</sup> Treatment is difficult with a low rate of full remission<sup>2</sup> thus highlighting the urgent need to identify early risk factors for prevention and targeted intervention. GAD is frequently undiagnosed and/or untreated in elderly people notably because of a focus of patients and practitioners on physical symptoms and the general assumption that high rates of anxiety are to be expected in elderly persons due to increased vulnerability.<sup>3</sup> As GAD prevalence peaks in middle age and appears to diminish in elderly people, it is also commonly assumed that older cases principally represent the continuing chronic course of an early-onset illness with very rare new onset in old age.<sup>4</sup> The drop in prevalence may, however, be because of poor case recognition in the elderly due to associated pathologies and differences in clinical presentation; the assumption being that the clinical characteristics of late-onset GAD and its risk factors are the same as for younger persons. Given that GAD is a risk factor for numerous chronic physical and mental disorders with high prevalence in the elderly, the identification of predictors specific to this age group is important for prevention and clinical management, thus having potentially significant consequences for overall health and daily functioning.<sup>3</sup>

Despite its prevalence and impact, knowledge about GAD onset in older people is still scarce, most studies having focused on

(young) adults and/or specific populations, and limited or particular risk factors (see Moreno-Peral *et al.*<sup>5</sup> for recent review). Most previous studies have been cross-sectional, which precludes differentiating between factors that co-occur with or result from psychopathology and etiological factors related to the occurrence of GAD. Only two prospective studies have evaluated the 3-year incidence and risk factors for GAD in older adults. Few candidate risk factors (mainly psychopathological) have been identified; history of depression and/or anxiety in the AMSTEL (Amsterdam Study of the Elderly),<sup>6</sup> and being female, posttraumatic stress disorder (PTSD) and narcissistic personality disorder in the NESARC (National Epidemiologic Survey on Alcohol and Related Conditions), the latter being the only study to examine comorbidity with other anxiety disorders.<sup>7</sup> The exposure window to risk factors has thus been very narrow precluding, for example, distal factors such as early or accumulated trauma, longstanding vulnerability with possible biological origins, as well as late-life events, such as age-related chronic and metabolic diseases and adverse life events.

To expand current knowledge of GAD in the elderly, the present study aimed at estimating the 12-year incidence of GAD and describing the predictors of incident GAD in late life in a large cohort of community-living elderly people, using a repeated standardized clinical interview for psychiatric disorder evaluation and a broad range of socio-demographic, lifestyle, biological and clinical risk factors, as well as early and recent adverse events.

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## MATERIALS AND METHODS

### Participants

Community dwelling persons 65 years and over were recruited by random selection from the 15 electoral rolls of the Montpellier district between March 1999 and February 2001 as part of the prospective cohort ESPRIT study of late-life psychiatric disorder.<sup>8</sup> Of the persons contacted, 72.7% accepted. Refusers were replaced by another subject drawn at random from the same electoral division such that each division is equally represented. Subjects refusing were slightly older and more likely to live alone than non-refusers. Each participant attended a half-day examination at inclusion and was re-examined with a detailed psychiatric interview on five further occasions at intervals of 2, 4, 7, 10 and 12 years. A flow chart is given as Supplementary Figure S1. Persons with dementia at baseline ( $n=70$ ) were excluded from the present study. Dementia was diagnosed by a neurologist as part of a standardized examination and validated by a panel of independent neurologists, as described previously.<sup>9</sup> Of the 2189 dementia-free participants included in the ESPRIT study, 215 were excluded because of missing data on GAD at baseline and 91 because of prevalent GAD. Of this sample, 172 participants were missing all follow-up examinations (33 died, 55 were lost to follow-up and 84 had no GAD data). The population incidence rate was evaluated on 1711 participants with data available for at least one of the five follow-ups. A further 245 subjects with missing data on covariates (for example, waist-to-hip ratio (7.8%) and visual impairment (6.7%); see Table 1) were excluded from the multivariate analyses leaving 1466 subjects in the final sample. The study protocol was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre and written informed consent was obtained from each participant.

### Psychiatric disorder assessment

The diagnosis of lifetime anxiety disorder (GAD, social phobia, specific phobia and agoraphobia, panic disorder, obsessive compulsive disorder and PTSD) and major depression were performed by psychologists and psychiatric nurses according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition) criteria and using the MINI (Mini-International Neuropsychiatric Interview; French version 5.00), as described previously.<sup>10</sup> The interviewers were initially trained for a 3-month period under the supervision of psychiatrists from the Department of Adult Psychiatry at Montpellier University Hospital. The MINI is a standardized and structured diagnostic examination validated within the general population setting,<sup>11</sup> which uses a nonhierarchical case-identification procedure, thus permitting the diagnosis of psychiatric comorbidities. GAD was established using the current definition implying the presence of symptoms for at least 6 months.<sup>4</sup> During the follow-up, MINI questions referred to the period since the previous examination, 2 or 3 years before. The positive cases were reviewed by a panel of independent psychiatrists as described previously.<sup>10</sup>

### Baseline socio-demographic, lifestyle, biological and clinical variables

The standardized interview included questions on socio-demographic characteristics (age, sex, education level ( $\geq 5$  years)), smoking (current versus ever), alcohol consumption ( $>12$  g per day), diabetes (glycemia  $\geq 7$  mmol l<sup>-1</sup> or treated), hypercholesterolemia (cholesterol  $\geq 6.2$  mmol l<sup>-1</sup> or treated), hypertension (resting blood pressure  $\geq 160/95$  mm Hg or treated), measures of weight, height, waist and hip, as well as binary clinical variables, for example, respiratory disorders, osteoporosis, thyroid disorder, cancer, physical activity. Body mass index (expressed as kg/m<sup>2</sup>) and waist-to-hip ratio were calculated. Detailed medical questionnaires (with additional information from general practitioners) provided information on history of ischemic pathologies (angina, myocardial infarction, stroke, cardiovascular surgery and arteritis) and nonischemic cardiac pathologies (arrhythmia and heart failure). The participants were asked to show medical prescriptions, drug packages and any other relevant information to record all past-month somatic and psychotropic medications taken. Exposure to adverse life events in the past year was assessed using the Gospel Oak questionnaire.<sup>12</sup> Mobility limitation, visual and hearing impairment were determined as described.<sup>13</sup> Venous blood samples were taken at baseline after 12-h fasting and lipid levels were measured.<sup>14</sup> Global cognitive function was measured using the Mini-Mental State Examination and a score  $<26$  was considered to be indicative of cognitive impairment.<sup>15</sup> Verbal fluency and visual memory were assessed by reference to Isaacs' Set<sup>16</sup> and the Benton Visual

Retention Test,<sup>17</sup> respectively. The Trail Making Tests A and B assessed psychomotor speed and executive function.<sup>18</sup> Low cognitive performance was defined as scoring in the lowest tertile except for the timed Trail Making Test (highest).

### Early environment

A self-report questionnaire<sup>19</sup> (with binary yes/no response categories) examining traumatic experiences during childhood and adolescence was completed by 1365 of the 1604 participants (85.1%) at the second follow-up assessment by which time the study interviewers had established close relationships, facilitating the request for sensitive information. The subjects having not completed this questionnaire were more likely to have cognitive impairment and mobility limitation ( $P < 0.01$ ) but did not differ regarding all the other characteristics including past GAD, incident GAD and other psychiatric disorders. It covered adverse exposure to severe abuse (physical, verbal or sexual abuse, neglect or excessive punishment), parental loss or separation, parents with mental disorder, alcohol or drugs problems, conflict at home, financial difficulties, excessive sharing of parental problems, war and natural catastrophe. Protective factors included parental affection, availability of an adult friend, having had a happy childhood or a normal education, parents perceived as doing their best, feeling happy at school and raised by both parents. Low affective support was defined as having reported less than six protective factors.

### Statistical analysis

Prevalent GAD cases were excluded to avoid a methodological bias related to reverse causality (impossibility of separating cause and effect over time). Chi-square tests compared the characteristics of participants included in the analyses with those excluded. The incidence rate over the 12-year follow-up was calculated for 1711 participants with no prevalent GAD at baseline and with data available for at least one of the five follow-ups. For the calculation of the incidence rate, a participant is counted only once as a case, irrespective of the number of successive episodes (events) he/she may have experienced during the follow-up, and the date of onset corresponded to the first episode. The exact date of onset during the follow-up period being potentially imprecise or not known, onset was therefore considered to have occurred midway between the two examinations. Population incidence was estimated by dividing the number of new cases that occurred during the follow-up by the total number of GAD-free years lived by the cohort from baseline, expressed as number of new cases per 1000 person-years. A Cox model with delayed entry was used in the longitudinal analysis of incident GAD. The proportional-hazards assumptions were tested using Martingale residuals. Multivariate models included baseline covariates meeting Martingale residual criteria for proportionality of risk and associated with incident GAD in Cox models adjusted for sex ( $P < 0.15$ ) and were reduced using a backward selection procedure keeping in the final model all the covariates significant at  $P < 0.15$  (model 1). Model 2 was further adjusted for past GAD. These models were performed with the subjects having no missing data on any covariates included in the most complete model. Additional analyses were performed with the participants without a history of past GAD ('first-onset cases') as well as, separately in those termed as 'recurrent' that is with past GAD. SAS (version 9.3, SAS Institute, Cary, NC, USA) was used for the statistical analysis and all tests were two-tailed, and the significance level was  $P < 0.05$ .

## RESULTS

### Baseline characteristics of the sample

Of the 2189 non-demented participants in the ESPRIT study, 215 were excluded because of missing data on GAD at baseline, as well as 91 participants with prevalent GAD, and a further 172 (9.1%) had missing data for follow-up (see Supplementary Figure S1). Compared with the 1711 participants included in the longitudinal analysis, the 478 excluded participants were significantly older with a lower education level and more frequently having ischemic pathologies ( $P=0.02$ ), respiratory disorders ( $P=0.004$ ), thyroid disorder ( $P=0.01$ ), as well as cognitive impairment, depression, anxiety disorder and more frequent psychotropic medication use ( $P < 0.0001$ ).

**Table 1.** Incident cases of GAD over 12-year follow-up according to baseline variables<sup>a</sup>

Characteristic	Total N	No GAD, N = 1568		Incident GAD, N = 143		P <sup>a</sup>
		N	%	N	%	
Age, years (mean, s.d.)	1711	72.6 (mean)	5.1 (s.d.)	72.4 (mean)	5.0 (s.d.)	—
<i>Socio-demographic characteristics</i>						
Sex (female)	1711	885	56.44	114	79.72	0.0001
Living alone <sup>b</sup>	1708	412	26.33	39	27.27	0.58
Childless	1624	151	10.15	15	11.03	0.95
Education level ( $\geq 5$ years)	1710	777	49.59	59	41.26	0.13
<i>Lifestyle</i>						
Alcohol consumption ( $> 12$ g per day)	1681	623	40.45	40	28.37	0.46
Smoking (current or ever)	1710	667	42.57	46	32.17	0.95
Physical activity	1513	563	40.62	45	35.43	0.37
BMI ( $\geq 25$ kg/m <sup>2</sup> )	1701	724	46.41	59	41.84	0.90
WHR ( $\geq 0.94$ )	1578	313	21.72	22	16.06	0.02
<i>Lifetime adverse events</i>						
Recent adverse events <sup>c</sup>	1667	872	57.11	97	69.29	0.005
<i>Childhood events</i>						
Severe abuse	1365	154	12.33	19	16.38	0.20
Parental loss or separation	1365	412	32.99	52	44.83	0.008
Parents with mental problems	1365	225	18.01	40	34.48	0.0005
Parents had problems with alcohol or drugs	1365	92	7.37	13	11.21	0.17
Conflict, nervous stress at home <sup>b</sup>	1365	195	15.61	29	25.00	0.05
Poverty, financial difficulties	1365	275	22.02	39	33.62	0.001
Parents too often sharing their problems with children	1365	164	13.13	21	18.10	0.17
Parent or adult friend affection	1365	1026	82.15	89	76.72	0.16
Low affective support	1365	174	13.93	28	24.14	0.005
War or natural catastrophe	1365	673	53.88	66	56.90	0.89
<i>Biological and clinical variables</i>						
LDL-cholesterol ( $> 4.01$ mmol l <sup>-1</sup> )	1688	500	32.30	38	27.14	0.06
HDL-cholesterol ( $< 1.73$ mmol l <sup>-1</sup> )	1698	1042	66.92	80	56.74	0.44
TG ( $\geq 0.95$ mmol l <sup>-1</sup> )	1698	1050	67.44	83	58.87	0.27
Hypercholesterolemia (cholesterol $\geq 6.2$ mmol l <sup>-1</sup> or treated) <sup>b</sup>	1702	863	55.32	92	64.79	0.34
Hypertension (resting blood pressure $\geq 160/95$ mm Hg or treated)	1711	695	44.32	64	44.76	0.10
Diabetes (glycemia $\geq 7$ mmol l <sup>-1</sup> or treated)	1697	134	8.61	14	9.93	0.12
Ischemic pathologies <sup>d</sup>	1711	220	14.03	13	9.09	0.83
Arrhythmia and heart failure	1705	198	12.67	23	16.20	0.04
Respiratory disorders (dyspnea, asthma, or bronchitis)	1711	73	4.66	14	9.79	0.0007
Osteoporosis	1696	273	17.54	41	29.29	0.18
Thyroid disorder	1700	111	7.13	14	9.79	0.89
At least one chronic disorder <sup>e</sup>	1711	973	62.05	89	62.24	0.21
MMSE ( $< 26$ ) <sup>b</sup>	1703	196	12.56	26	18.31	0.03
Isaacs Set test score (lowest tertile)	1682	394	25.52	42	30.43	0.009
Benton Visual Retention Test score (lowest tertile)	1695	312	20.09	34	23.94	0.05
Trail Making Test A score (highest tertile)	1686	419	27.12	48	34.04	0.003
Trail Making Test B score (highest tertile)	1641	421	27.97	45	33.09	0.02
Visual impairment	1597	88	6.02	11	8.21	0.10
Hearing impairment	1703	64	4.10	4	2.80	0.88
Mobility limitation	1705	59	3.78	8	5.59	0.06
Number of somatic medications $\geq 4$	1711	698	44.52	75	52.45	0.01
<i>Mental health</i>						
Use of psychotropic medication	1711	186	11.86	26	18.18	0.03
Major depression	1698	24	1.54	10	7.04	0.0001
Anxiety disorder (without GAD)	1701	141	9.04	29	20.42	0.0003
Phobia	1702	133	8.53	27	18.88	0.001
Posttraumatic stress disorder	1711	2	0.13	1	0.70	NA <sup>f</sup>
Panic disorder	1710	3	0.19	0	0.00	NA <sup>f</sup>
Obsessive compulsive disorder	1711	6	0.38	1	0.70	NA <sup>f</sup>
Past GAD	1711	85	5.42	29	20.28	0.0001

Abbreviations: BMI, body mass index; GAD, generalized anxiety disorder; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMSE, Mini-Mental State Examination; TG, triglycerides; WHR, waist-to-hip ratio. <sup>a</sup>Cox model with delayed entry adjusted for age as time scale and sex (except when sex was examined).

<sup>b</sup>Variables not meeting Martingale residual criteria for proportionality of risk. <sup>c</sup>At least one recent adverse event during the past year. <sup>d</sup>Ischemic pathologies correspond to angina pectoris, myocardial infarction, stroke, cardiovascular surgery and arteritis. <sup>e</sup>Chronic disorders correspond to hypercholesterolemia, hypertension, diabetes, asthma, osteoporosis, thyroid disorder and recent cancer. <sup>f</sup>Not applicable (NA) due to the low number of cases.

**Table 2.** Multivariate Cox Model with delayed entry of incident GAD over 12-year follow-up ( $n = 1466$ , 125 cases)<sup>a</sup>

Characteristic	Model 1			Model 2		
	HR	95% CI	P	HR	95% CI	P
Sex (female)	3.38	1.93–5.90	< 0.0001	3.17	1.81–5.55	< 0.0001
Waist-to-hip ratio (WHR $\geq 0.94$ )	1.83	0.98–3.44	0.060	1.86	0.99–3.50	0.054
LDL-cholesterol ( $> 4.01 \text{ mmol l}^{-1}$ )	0.62	0.42–0.94	0.023	0.60	0.40–0.90	0.013
Arrhythmia and heart failure	1.67	1.04–2.70	0.035	1.72	1.07–2.77	0.027
Respiratory disorders (dyspnea, asthma or bronchitis)	2.81	1.57–5.03	0.0005	3.02	1.68–5.41	0.0002
Isaacs Set test score (lowest tertile)	1.49	1.00–2.23	0.048	1.50	1.00–2.23	0.048
Recent adverse events	1.73	1.17–2.55	0.006	1.64	1.11–2.42	0.013
Use of psychotropic medication	1.58	1.00–2.50	0.049	1.64	1.03–2.59	0.036
Current major depression	3.47	1.75–6.90	0.0004	3.55	1.76–7.12	0.0004
Current phobia	2.26	1.43–3.56	0.0005	1.93	1.21–3.06	0.006
Past GAD				4.06	2.63–6.26	< 0.0001

Abbreviations: CI, confidence interval; GAD, generalized anxiety disorder; HR, hazard ratio; LDL, low-density lipoprotein. <sup>a</sup>Multivariate models included baseline covariates meeting Martingale residual criteria for proportionality of risk and associated with incident GAD in Cox models adjusted for age and sex ( $P < 0.15$ ) and were reduced using a backward selection procedure keeping in the final model all the covariates significant at  $P < 0.15$ .

The baseline characteristics of the participants included in the analyses are shown in Table 1. The mean (s.d.) age was 72.6 (5.1) years with 58.4% women. The prevalence of major depression at baseline in the sample was 2.0% and that of phobia was 9.4%. PTSD and panic disorder each accounted for < 0.2% and obsessive compulsive disorder for 0.4%. Psychotropic medication was taken by 12.4% of the participants, antidepressant for 7.2%; anxiolytics for 3.0 and 2.2% took both of them.

#### Risk factors for incident GAD

The median (interquartile range) duration of the follow-up was 9.7 (7.4) years. Over the 12-year follow-up, 143 of the 1711 participants (8.4%, 95% confidence interval (CI) = 7.1–9.7%) without GAD at baseline reported a new episode of GAD, being the first onset for 80%. The median (interquartile range) age at incident GAD diagnosis was 74.8 (7.9) years and the median (interquartile range) time of diagnosis from baseline examination was 2.6 (2.2) years. The median (interquartile range) age of first onset for recurrent cases was 40 (28) years. The estimated incident rate was 10 per 1000 person-years. Multivariate Cox models with delayed entry were performed with the subjects having no missing data on the covariates included in the complete model 2. In multi-adjusted model 1 using backwards selection removal criteria at  $P < 0.15$ , being female, having respiratory disorders, arrhythmia and heart failure, lower Isaacs score, current depression, phobia, reporting recent adverse life events and using psychotropic medication, were significantly associated with incident GAD, whereas high low-density lipoprotein (LDL)-cholesterol decreased the risk (Table 2). A marginal positive association was also observed with high waist-to-hip ratio. The same associations were found after further adjustment for past GAD, which was also highly significantly associated with incident GAD (model 2).

The same results were found when restricting the analyses to subjects with a first episode of GAD (that is, without a history of past GAD, Table 3), except for the use of psychotropic medication ( $P = 0.23$ ). On the other hand, for the participants with recurrent GAD ( $n = 28$ ), only sex (hazard ratio (HR) = 3.55, 95% CI = 1.21–10.41,  $P = 0.02$ ), major depression (HR = 5.82, 95% CI = 1.32–25.71,  $P = 0.02$ ) and psychotropic medication (HR = 2.36, 95% CI = 1.00–5.60,  $P = 0.05$ ) were associated with occurrence of a new episode and no significant associations were found with phobia (HR = 2.19, 95% CI = 0.87–5.54,  $P = 0.10$ ), recent trauma (HR = 1.92, 95% CI = 0.84–4.40,  $P = 0.12$ ) or any chronic disorder ( $P > 0.35$ ).

**Table 3.** Multivariate Cox Model with delayed entry for first onset of incident GAD ( $n = 1367$ , 97 cases)<sup>a</sup>

Characteristic	HR	95% CI	P
Female gender	3.43	1.83–6.41	0.0001
Waist-to-hip ratio (WHR $\geq 0.94$ )	1.89	0.93–3.85	0.077
LDL-cholesterol ( $> 4.01 \text{ mmol l}^{-1}$ )	0.50	0.31–0.82	0.006
Arrhythmia and heart failure	1.91	1.15–3.18	0.013
Respiratory disorders (dyspnea, asthma or bronchitis)	2.95	1.57–5.55	0.0008
Isaacs Set test score (lowest tertile)	1.61	1.03–2.50	0.035
Recent adverse events	1.72	1.11–2.68	0.016
Current major depression	3.60	1.69–7.67	0.0009
Current phobia	2.55	1.51–4.30	0.0005

Abbreviations: CI, confidence interval; GAD, generalized anxiety disorder; HR, hazard ratio; LDL, low-density lipoprotein. <sup>a</sup>Multivariate models included baseline covariates meeting Martingale residual criteria for proportionality of risk and associated with first onset of incident GAD in Cox models adjusted for age and sex ( $P < 0.15$ ) and were reduced using a backward selection procedure keeping in the final model all the covariates significant at  $P < 0.15$ . Psychotropic medications initially included were not retained in the final model ( $P = 0.23$ ).

#### Impact of childhood environment on incident GAD

Of the 1173 non-demented participants having completed the childhood questionnaire and free of prevalent GAD and with no missing variables for covariates, 104 had incident GAD during the 12-year follow-up. In fully adjusted Cox model 1 with backwards selection removal criteria at  $P < 0.15$ , significant associations were found for parental loss or separation (HR = 1.58, 95% CI = 1.06–2.34,  $P = 0.02$ ), parents with mental problems (HR = 1.75, 95% CI = 1.16–2.63,  $P = 0.007$ ), financial difficulties (HR = 1.65, 95% CI = 1.08–2.52,  $P = 0.02$ ) and low affective support (HR = 1.77, 95% CI = 1.12–2.80,  $P = 0.01$ ) independent of the other factors.

#### DISCUSSION

In this large prospective study in community-dwelling elderly, 8.4% (95% CI = 7.1–9.7%) of the participants without GAD at baseline developed GAD over 12 years; the incident rate being 10 per 1000 person-years. This was a first episode for 80% of cases. A large range of risk factors of late-life incident GAD were identified; being female, reporting recent and childhood adverse events, having chronic physical and mental health disorders. Most of these factors have not been previously reported in the elderly. In

the AMSTEL study, 3.9% of the participants without baseline psychopathology developed GAD over 3 years (estimated incident rate was 12 per 1000 person-years) and only a personal history of depression and/or anxiety was significantly associated with incident GAD symptoms, and decline in incapacity for activities of daily living was specific to GAD comorbid with depression.<sup>6</sup> In the NESARC study, 1.6% were new cases of GAD over 3 years (estimated incident rate was 5 per 1000 person-years) and the predictors were being female, narcissistic personality, and PTSD, whereas no significant associations were found with major depression or phobia.<sup>7</sup> Both of these studies were limited by only one follow-up examination over 3 years with thus a lower number of incident cases and statistical power. None of them examined psychotropic medication, early environment and chronic or metabolic disorders, nor did they differentiate recurrent from first-episode GAD.

In our study, major depression, phobia and past GAD were independent risk factors for incident GAD. Depression and female gender were observed to be risk factors for both first-onset and recurrent GAD, whereas phobia was a significant risk factor for first-onset GAD only, however, the low number of recurrent cases precludes drawing definite conclusions. Taking psychotropic medication was associated with recurrent GAD but not with first-onset GAD despite a >3-fold higher number of cases, which may reflect a low efficacy of medications in preventing GAD relapse. However, the lack of information regarding medication indication and prescriptions precluded definite conclusions. The number of cases of other anxiety disorders, especially PTSD and panic disorder, was very low in this elderly sample ( $n=3$ , cf. Table 1) and they were thus not examined. Their low prevalence suggests that they are unlikely to be significant risk factors.

A key finding from this study is that first episodes of GAD in late life are more common than previously believed and are related to specific risk factors, including environmental, intrinsic as well as extrinsic factors, notably age-related chronic disorders (respiratory disorders, arrhythmia and heart failure), lipid levels, adiposity and cognitive impairment. Stress has a significant role in the etiology of these disorders, and they are also known in themselves to generate chronic stress. Conversely, dysfunction of the autonomic nervous system and hypothalamic–pituitary–adrenal (HPA) axis has been reported in GAD.<sup>20,21</sup> Reduced lung function, asthma and chronic obstructive pulmonary disease have been associated with prevalent GAD<sup>22,23</sup> and clinical studies on pulmonary rehabilitation treatments have been shown to reduce anxiety symptoms.<sup>24</sup> There is some evidence of shared neural substrates for HPA and the respiratory control system with bidirectional connections having been reported for dyspnea.<sup>25</sup> Heart failure and arrhythmia are also considered as stress-related diseases associated with dysregulation of autonomic nervous system and HPA axes.<sup>26,27</sup> A recent case–control study in young adults reported an association between worry, the cardinal symptom of GAD, and a diminished heart rate stress response independent of GAD, with a possible suppression of adrenergic sympathetic stress responses in GAD specifically.<sup>28</sup>

In response to chronic stress, the de-regulation of the autonomic nervous system and HPA axis could lead to metabolic alterations.<sup>27,29</sup> In our study, lipid levels and adiposity were associated with GAD differently. The fact that higher abdominal obesity but not general body mass was a risk factor for incident GAD is consistent with an over-reactivity of the HPA axis. On the other hand, high LDL-cholesterol but not ischemic or vascular pathologies were associated with decreased GAD incidence, which may be consistent with neural mechanisms. Controversial findings have been found in cross-sectional studies with nonsignificant, positive or negative associations with cholesterol.<sup>30</sup> A few small studies showed an inverse association between anxiety and LDL-cholesterol in young adults.<sup>31,32</sup> LDL-cholesterol is the major carrier of cholesterol, notably required for

the regulation of cell membrane viscosity. Increase in serum LDL-cholesterol could be associated with increased brain cell membrane cholesterol, and changes in density and functioning of neurotransmitter transporters or receptors.<sup>30</sup> We have already reported a negative association and interaction with serotonin transporter for late-life depression<sup>14</sup> and experimental studies suggested that cholesterol may influence cholecystokinin and GABA receptors.<sup>33</sup>

Cognitive function was previously examined using Mini-Mental State Examination in two prospective studies, the AMSTEL study on GAD<sup>6</sup> and the Longitudinal Aging Study Amsterdam on anxiety symptoms,<sup>34,35</sup> showing no significant associations. A few small case–control studies supported an association between GAD and deficits in cognitive control (that is, inhibitory control in interference task, processing speed and shifting of attention in the Trail Making Test, verbal fluency).<sup>36</sup> In our study, performance on the Trail Making Test and Mini-Mental State Examination were also associated with incident GAD in the Cox model only adjusted for sex (cf. Table 1) but not in multivariate models. Verbal fluency gave the most significant and robust data, and was the only task specifically associated with cases of GAD occurring after 50 years of age.<sup>37</sup> The directionality between anxiety and cognitive control is currently uncertain; our results indicating that pre-existing cognitive deficits, notably tests depending on prefrontal processing, increase the risk of late-onset GAD.

A final noteworthy finding from our study is that in contrast with the AMSTEL<sup>6</sup> and NESARC<sup>7</sup> studies, exposure to adverse events, both recent and distal (more than 50 years before), were independently associated with incident GAD. Lifetime threatening events have been associated with the onset of GAD in young adults.<sup>5</sup> Two cross-sectional studies did not find significant associations between prevalent GAD in elderly people and recent or early adverse events, for example, sexual and physical abuse, parental loss and neglect.<sup>38,39</sup> In our study, poverty, parental loss or separation and low affective support were significantly associated with incident GAD. Negative parenting behavior and insecure attachment have already been associated with GAD in children and young adults.<sup>40,41</sup> Exposure to stressful events has been associated with CNS dysfunction and marked long-term changes in brain circuitry regulating stress reactivity involving the HPA axis.<sup>42</sup> We have already reported in this cohort that lifetime GAD was associated with increased secretion of cortisol under stress conditions.<sup>21</sup> We also found an association between early adverse events and worse verbal fluency,<sup>43</sup> as well as between cortisol levels and verbal fluency.<sup>44</sup> Interestingly, in randomized controlled trials, SSRI antidepressants have been reported to improve both GAD symptomatology and also neuropsychological functioning, associated with a decline in cortisol and cognitive improvement.<sup>45–47</sup> Whether the HPA axis could act as a mediating factor between stressful events and GAD remains to be examined. In the present study, we also found that a history of mental disorder in parents increased the risk of incident GAD as also reported in younger cohorts.<sup>5</sup> This could reflect both early shared environment and genetic vulnerability to anxiety disorder, considering the 30% heritability of GAD and familial link between subtypes of anxiety disorders.<sup>48</sup>

Limitations to this study should be considered when interpreting the results. Selection bias concerned the recruitment from electoral rolls, the response rate, and the exclusion of institutionalized elderly people, which limits the extent to which these findings can be generalized to the wider community of older adults as study volunteers tend to be younger, better educated and healthier than the overall population. The exclusion of some participants with missing data is also a potential source of bias, these people being older with lower educational level and worse physical and mental health, and thus more likely to be diagnosed with GAD. Although the loss during the 12-year follow-up period was low for an epidemiological study in elderly people and

physical illness well represented in this sample, we could not exclude bias due to loss to follow-up of a more disabled group, which may have led to an underestimation of the actual number of cases and also reduced the overall power of the study. This may also limit the generalizability of our results, and associations may have thus been underestimated. A further limitation was that some of the covariates were self-reported and retrospective (notably for life events especially during childhood) and may have been subject to recall bias with GAD participants responding more negatively about their health. However, similar associations were generally seen in the unadjusted and adjusted analysis, suggesting this is to be unlikely. Participants diagnosed with probable/possible dementia at inclusion were excluded from this analysis to minimize recall bias. However, as such individuals may also have higher rates of anxiety symptoms this could decrease the overall power of the study, possibly underestimating the associations found. Despite extensive adjustments, the possibility remains that unmeasured factors such as other social environment and personality traits may also be involved and confound the associations. Finally, since multiple analyses have been performed we cannot exclude that some associations were due to chance. However, many of the associations reaching traditional significance levels remained significant even after applying overly conservative multiple testing correction.

Conversely, this prospective study is based on a large sample representative of community-dwelling elderly people with five follow-up waves over 12 years, which enhances the accuracy and provides sufficient stability of incidence rate estimates. Extensive information was obtained on clinical status and medication (notably psychotropic medication), which was verified by examining prescription and medications, thus minimizing exposure misclassification. We were able to obtain differential diagnosis of specific anxiety disorders using a standardized psychiatric examination on the basis of DSM-IV criteria with clinical validation of the cases, thus minimizing false positive. Diagnosis was assessed by trained staff (psychologists and psychiatric nurses), which also allowed minimizing false negative. The exact date of GAD event was not always known and the onset was considered to have occurred midway between two assessments to minimize potential recall bias. In contrast with previous studies, we controlled for a large number of potential confounders, particularly lifestyle, early and recent adverse events, measures of physical and mental comorbidity and history of GAD (with a possible risk of over-adjustment), and we could also evaluate predictors of first late-onset GAD specifically.

To our knowledge, this is the most comprehensive prospective study in the general elderly population to date, providing novel information on the incidence and predictors of late-onset GAD over 12 years. Contrary to what is commonly believed, a significant number of cases occur for the first time in late life with specific age-related predictors thus supporting a vulnerability/stress model for onset. Our study suggests longstanding vulnerability with possible biological origins involving stress systems, such as metabolic disorders (adiposity), chronic diseases (respiratory disorders, arrhythmia and heart failure, cognitive impairment) and environment traumatic factors, including early events. GAD appears as a multifactorial stress-related affective disorder. Some of the risk factors identified in this study can be modified by socio-political intervention (childhood environment) or by health care intervention (physical or mental health problems). The identification of psychopathological, health- and stress-related risk factors could be essential for the early identification and treatment of late-life GAD, which is frequent but often undiagnosed, thus having potential major health and socio-economic consequences by decreasing comorbidity, disability and mortality.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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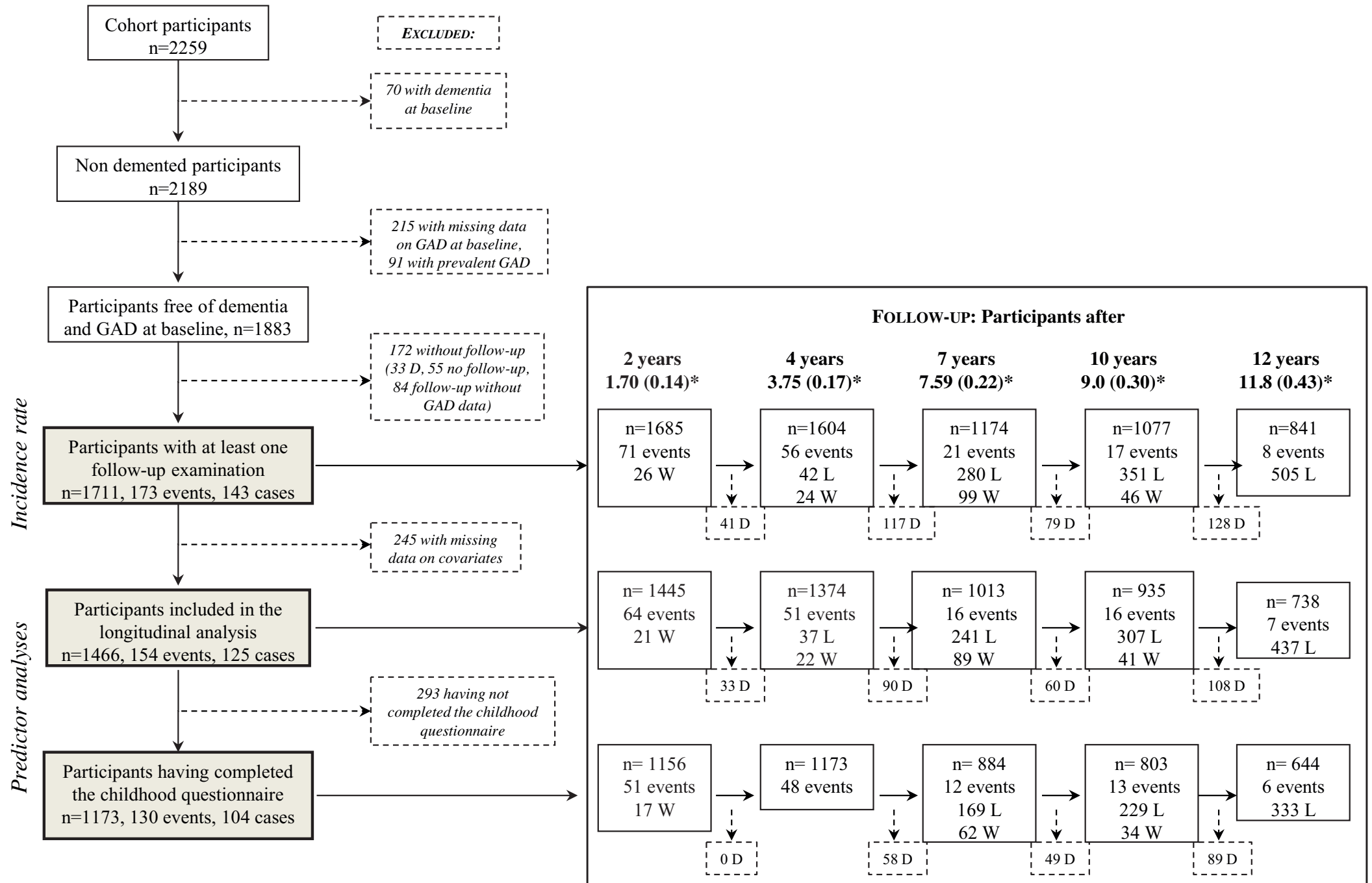
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Supplementary Information accompanies the paper on the Translational Psychiatry website (<http://www.nature.com/tp>)

# **BASELINE CHARACTERISTICS:**



**Fig. S1: Study flow chart**

D: died, L: lost all follow-ups, W: temporary withdrawal from follow-up; \* Median (IQR) duration of each follow-up (expressed as years).

## **2.3 Further evidence for a role of the adrenergic nervous system in generalized anxiety disorder**

## **SECTION 5 – DISCUSSION**

## CHAPTER 1 - Prevalence and incidence rates of late-life GAD

In this large prospective study in community-dwelling elderly, we observed a 6-month current GAD prevalence of 4.6% (95%CI= 3.7-5.5%) and the rate was 2.2-fold higher in women than in men (6% vs. 2.7%, respectively). Whereas a sex ratio around 2 is reliably reported, the prevalence rates of GAD in this sample appeared somewhat higher than those reported in other epidemiological studies in general populations (see Tables 1 and 3). This may be attributed to a number of methodological differences, concerning reference periods, heterogeneity of samples regarding age, sex, ethnicity, data collection method, diagnostic tools, and classification. In particular, diagnostic systems such as DSM-III and GMS/AGECAT contain explicit diagnostic criteria but their validity in relation to anxiety disorder in the elderly has been questioned on the grounds of their variable use of hierarchies and reasonableness criteria and also the differing concepts of what constitutes anxiety disorders. The GMS/AGECAT system allows a diagnostic on a hierarchical basis with anxiety disorders at the bottom; similarly, DSM-III has exclusion criteria so that a diagnosis of an anxiety disorder cannot be made if another condition such as major depression is present. Given the comorbidity of anxiety disorders with other diagnoses, this may lead to an underestimation of the true rate and obscure certain associations (Lindesay et al., 2004).

In the Esprit study, we used the MINI which is a standardized clinical interview based on DSM-IV criteria with a clinical validation of cases allowing an accurate evaluation of prevalence rates. It should be noted that the same current prevalence rates (4.4% and a female to male ratio of 1.9) were recently reported in a French sample of 5,600 adults using MINI and DSM-IV criteria (Malard et al., 2015). These rates are higher than those reported elsewhere in elderly (between 1.2 and 2.9% using DSM-IV criteria, see Tables 1 and 3). Few large epidemiological studies have reported lifetime prevalence of GAD in older adults (see Table 1). In our sample, lifetime prevalence (11%) was also high compared with the 2.8% reported in the NESARC study (Reynolds et al., 2015). This could result from the fact that this elderly population had been exposed to several successive wars between 1914 and 1962; the First World War for the oldest, the Second World War for all of them, the war in Indochina for a small proportion, and in Algeria for more than a quarter of our population (Ritchie et al., 2004). Also supporting this hypothesis is the finding that all current cases of GAD except one were recurrent and the median age (IQR) of first onset was 35 (28) years, that is 38 years before their inclusion in 1999 (76 years before, for the lowest quartile).

During the 12-year follow-up, 8.4% (95%CI= 7.1-9.7%) of the participants without GAD at baseline experienced incident GAD, and the incidence rate was estimated to be 10 per 1000 person-years. Contrary to what is commonly believed, a significant number of cases (80% of the incident cases) occur for the first time in late life (20% had past GAD). Up to now, only two prospective studies have examined incident GAD in the elderly general population. In the Dutch AMSTEL study, 3.9% of the participants developed GAD at follow-up and the estimated incident rate (12 per 1000 person-years) was comparable to what we observed, whereas in the American NESARC study the incidence rate was lower (5 per 1000 person-years), only 1.6% being new cases. Both studies were limited by only one follow-up examination over 3-years. Our study had five follow-up waves over 12 years and in contrast with the AMSTEL study, a greater number of incident cases and a standardized psychiatric examination was used according to DSM-IV criteria.

## CHAPTER 2 – Current psychiatric comorbidity with GAD

High levels of comorbidity have been reported between GAD and major depression throughout the life (Axelson et al., 2001; Blanco et al., 2014; Simon, 2009). Several studies suggest that GAD could possess substantial overlap with major depression regarding heritability and certain risk factors (e.g. childhood adverse events, demographics characteristics,...) (Hettema, 2008). Some investigators have thus questioned whether GAD could be a disorder clinically distinct from depression or just be a severity marker of other psychiatric disorders (Beesdo et al., 2010). In the Avon Longitudinal Study of Parents and Children, Davies *et al.* recently reported that GAD was not a consequence of depression but an etiologic factor contributing to depression, the symptoms of GAD at 15 years of age being able to predict depression at 18 years (Davies et al., 2016). Three large prospective studies in children and young adults also indicate that despite both disorders sharing some common risk factors, the etiological pathways may differ (Beesdo et al., 2010; Kessler et al., 2008; Moffitt et al., 2007). A systematic review indicated that despite substantial overlap, GAD tended to differ from depression. For example, there are differences regarding sleep characteristics, with reduced sleep efficiency and total sleep in GAD, but rapid eye movement sleep disturbances in depression (Hettema, 2008). In adults, Blanco *et al.* also suggest that GAD and major depression are closely related, but constitute different nosological entities with distinct latent structures, clinical manifestations, and patterns of comorbidity (Blanco et al., 2014). In the elderly, the LASA study reports that the risk factors for pure major depression are younger age and external locus of control, whereas a wide range of factors (lower education level, chronic physical illness, functional limitation, small network, emotional support, stress and locus of control) are associated with pure GAD. Hence, the risk factors for pure GAD and pure depression appear more different than similar, external locus of control being the only common factor (Beekman et al., 2000). In our elderly sample, the current prevalence of major depression was 3% and only 14.4% (n=13) of late-life GAD cases were comorbid with major depression. Although we could not compare the characteristics of GAD and depression, some well-known factors associated with late-life major depression (age, cardiovascular pathologies, smoking, and physical activity (Vink et al., 2008)) failed to be significantly associated with GAD. In addition, neither adjustment for major depression, nor exclusion of depressed participants in sensitivity analyses changed the significant findings indicating that the associations were not confounded by depression. Together, this suggests that late-life GAD and major depression are distinct. Our data also showed that late onset GAD occurring after 50 years of age (25% of all GAD cases) may have some specific age-related characteristics, especially worse verbal fluency, compared with early onset ( $p=0.006$ ).

Regarding the comorbidity with other anxiety disorders, nearly 35% of the GAD cases were comorbid with phobias of all types, less than 5% were comorbid with OCD or PTSD, and none with panic disorder. Some factors were common to both “pure” GAD and “pure” phobia, such as female sex, impaired verbal fluency, and depression. On the other hand, younger age and higher education level were significantly associated with a lower risk of “pure” phobia specifically, whereas BMI, somatic and psychotropic medications were specific to “pure” GAD. The large MHGP study in French adults found a higher prevalence of GAD (12.8%) but a lower rate for phobia (6.4%) (Leray et al., 2011). As in our elderly cohort, they found sex and depression to be associated with both GAD and phobia, but also younger age, which was only specific to phobia in our elderly cohort. They did not examine physical health or psychiatric comorbidity. All these results suggest that GAD may have specific characteristics, notably distinct from phobia.

## CHAPTER 3 – Use of psychotropic medications

In our sample, only 36.3% of the current GAD cases were taking psychotropic medications; with the treatment preference being for anxiolytics taken alone by 42.5% of the cases and in combination with antidepressants by 24%, whereas one third were taking only antidepressants. All the prevalent GAD cases but one were recurrent. Nearly two thirds of the GAD cases received no treatment which suggests under-recognition and/or inappropriate treatment in the elderly. This conclusion was consistent with a survey in six European countries showing that less than 25% of the participants with a diagnosis of major depression or anxiety disorder reported adequate treatment in the general medical care sector (Fernandez et al., 2007). In a primary care survey in Germany, only 34% of the actual GAD cases were correctly diagnosed as GAD, and only 20% were receiving treatment (Wittchen et al., 2002). Taking psychotropic medication could receive negative public attention, and patients may feel judged and embarrassed (Boyd et al., 2015). Most adults have high self-stigma when taking these medications and this may increase the psychological burden (Gaudiano et al., 2013). Thus, drug compliance could be poor, and patients reluctant to get the full course of treatment, contributing to the chronic evolution of the disease. In the USA, nearly one in four individuals believes that psychiatric medications are harmful to the body, and approximately 1 in 3 believes that medications interfere with one's daily activities (Croghan et al., 2003). In addition, high comorbidity with other chronic disorders is common in the elderly, suggesting greater attention may be paid to side effects and interactions with other medications. All these potential reasons may discourage elderly persons from seeking treatment when needed, and may bias their appraisal of treatment efficiency, thus leading to an increased prevalence of GAD.

## CHAPTER 4 – Factors associated with late-life GAD

### 4.1 General characteristics of late-life GAD

We examined a large range of potential risk factors for GAD, namely socio-demographic, lifestyle, biological, lifetime adverse events, as well as physical and mental health characteristics. **Table 7** summarizes the risk factors that were found to be independently and significantly associated with late-life GAD in our cross-sectional or longitudinal analyses.

**Table 7.** Summary of risk factors associated with prevalent and 12-year incident GAD in the Esprit study

Characteristic	Prevalent GAD		Incident GAD	
	N=1825, 84 events		N=1466, 125 events	
	OR [95%CI] <sup>1</sup>	p	HR [95%CI] <sup>1</sup>	p
Sex (female)	NS <sup>2</sup>		3.17 [1.81-5.55]	<0.0001
Body mass index (BMI $\geq 25$ kg/m <sup>2</sup> )	0.59 [0.35-0.98]	0.04	NS	
Waist-to-hip ratio (WHR $\geq 0.94$ )	NS		1.86 [0.99-3.50]	0.054
LDL-cholesterol (> 4.01 mmol/l)	NS		0.60 [0.40-0.90]	0.013
Arrhythmia & heart failure	NS		1.72 [1.07-2.77]	0.027
Respiratory disorders	NS		3.02 [1.68-5.41]	0.0002
Cognitive impairment (verbal fluency)	NS <sup>2</sup>		1.50 [1.00-2.23]	0.048
Nb. of somatic medications $\geq 4$	1.61 [0.99-2.60]	0.06 <sup>2</sup>	NS	
Use of psychotropic medication	2.62 [1.56-4.40]	0.0003	1.64 [1.03-2.59]	0.036
Current major depression	2.80 [1.29-6.11]	0.01	3.55 [1.76-7.12]	0.0004
Current phobia	3.72 [2.22-6.23]	<0.0001	1.93 [1.21-3.06]	0.006
Past GAD	NA		4.06 [2.63-6.26]	<0.0001

Recent adverse events	NS		1.64 [1.11-2.42]	0.01
Childhood events:				
Low affective support	1.98 [1.00-3.91]	0.05	1.77 [1.12-2.80]	0.01
Parental loss or separation <sup>3</sup>	ND		1.58 [1.06-2.34]	0.02
Parents with mental problems <sup>3</sup>	ND		1.75 [1.16-2.63]	0.007
Poverty, financial difficulties <sup>3</sup>	ND		1.65 [1.08-2.52]	0.02

<sup>1</sup> OR and HR obtained in the most complete multivariate models (M2).

<sup>2</sup> Significant associations were found with pure GAD without phobia for sex (OR= 2.20, 95%CI= 1.15-4.21, p=0.02) and cognitive impairment (OR=1.86, 95%CI=1.05-3.30, p=0.03).

<sup>3</sup> N=1173, 104 incident events.

NA: not applicable (all prevalent cases but one having a history of GAD); ND: not determined; NS: not significant.

Reporting low affective support during childhood, and mental health factors (diagnoses, medication) were associated with both prevalent and incident GAD. Some chronic physical disorders (respiratory disorders, arrhythmia and heart failure, and dyslipidemia) were associated with incident but not with prevalent GAD but the global indicator of physical health (number of somatic medications >4) was marginally associated with prevalent GAD (and this was significant for pure GAD). Being female and cognitive impairment were associated with both pure prevalent and incident GAD. Recent stressful events were significantly associated with incident but not prevalent GAD. These differences could be due to a lower statistical power, the number of events in the longitudinal analyses being 50% greater than in the cross-sectional analyses. In contrast, adiposity showed a distinct pattern; a high BMI was associated with a lower risk of prevalent GAD whereas high WHR was associated with a higher risk of incident GAD.

A key finding is that first episodes of GAD in late life are more common than previously believed and are related to specific risk factors compared to recurrent cases. Female gender and major depression are common risk factors for both first-onset and recurrent GAD. Current phobia, high LDL-cholesterol, respiratory disorders, recent trauma, arrhythmia and heart failure are specifically associated with first-onset GAD, whereas taking psychotropic medication is significant for recurrent GAD only. This could reflect higher psychiatric comorbidity and chronicity of GAD and a lower efficacy of psychotropic medications in preventing relapse in the elderly (as also suggested by the high rate of recurrent cases). Feltner *et al.* treated 624 adults who had experienced GAD for at least one year, and found that a 24-week pregabalin treatment course could significantly slow-down or prevent relapse compared to placebo (Feltner *et al.*, 2008). Pregabalin marketing authorization (AMM) is recent (2015); consequently we were not able to examine this with our data set. Although understanding and treatment of GAD have been improved, the levels of relapse remain high which calls for a deeper knowledge of the etiologic factors of late-life GAD.

The only two previous prospective studies in elderly general populations were limited by a short follow-up with only one examination over 3-years and low statistical power. In the AMSTEL study, using GMS-AGECAT, having a personal history of depression or anxiety was the only factor

significantly associated with the onset of GAD, whereas a decrease in instrumental daily activity functioning was specific to GAD comorbid with depression (Schoevers et al., 2005). In the NESARC study female gender, a history of PTSD, and narcissistic personality were the only significant risk factors for GAD (Chou et al., 2011). Several longitudinal studies in adolescents and young adults also report associations with adverse family environment and personality profiles but these studies were not extended to older adults (Beesdo et al., 2010; Grant et al., 2009). None of the previous studies examined past GAD (recurrent episodes), psychotropic medications, and chronic or metabolic disorder. Our study filled in some gaps and overcame some of the limits of previous studies notably in providing accurate information on childhood adverse events as well as metabolic or chronic disorders.

## **4.2 Socio-demographic and lifestyle characteristics**

In our study, female gender was associated with an increased risk of prevalent and incident GAD as generally reported in adults and the elderly (Ansseau et al., 2008; Chou et al., 2011; Grant et al., 2009; Grant et al., 2005; Leray et al., 2011; Lim et al., 2005; Mackenzie et al., 2011; Schoevers et al., 2003). Conversely, no significant associations were found with incident late-life GAD for education and marital status as also reported in the elderly (Chou et al., 2011; Goncalves et al., 2011; Schoevers et al., 2005) as well as for physical activity, which had not been evaluated before in the elderly. Very few studies have examined tobacco and alcohol consumption in the elderly. No significant associations were found with prevalent and incident GAD in our study as reported in the cross-sectional ANSMHWB and longitudinal NESARC studies (Chou et al., 2011; Goncalves et al., 2011).

Limited research has been conducted on the association between GAD and BMI. The American NESARC study reported a significant association between being obese or overweight and prevalent GAD (Barry et al., 2008; Petry et al., 2008), but one cannot exclude that the use of psychoactive medications may also affect weight. Conversely, another study showed that constitutionally thin men were more than twice as likely to have GAD than controls (Mazzeo et al., 2004). One hypothesis is that GAD patients may experience the anxiolytic effects of excessive exercise and fasting and be more likely to engage in behaviors that favor lower BMI (Thornton et al., 2011). In our study, the negative association between BMI and GAD was independent of sociodemographic, physical and mental health variables, including the use of psychotropic medication, but no significant associations were found with incident GAD. This may be explained by reverse causality, those with prevalent GAD having a lower BMI as a result of symptoms and declining health. This is also supported by, a prospective study in young adults which reported a negative association between GAD and being overweight after a 20-year follow-up (Hasler et al., 2004).

## **4.3 Stressful life events**

Lifetime exposure to stressors and sensitivity to stress have been shown to be involved in anxiety but their consequences have rarely been investigated in late-life GAD (Moreno-Peral et al., 2014). Several retrospective and prospective studies have found that maltreatment, neglect, severe abuse as well as parental separation or loss were associated with the onset of GAD in young and adult populations (Beesdo et al., 2010; Chou, 2012; Cougle et al., 2010; Kessler et al., 2008; Moffitt et al., 2007). More recent stressful life events were also associated with prevalent GAD in adults (Lim et al., 2005; Muhsen et al., 2008). Only two cross-sectional studies have been performed in the elderly; a significant association with recent interpersonal losses or extreme experience during World War II was reported in the LASA (Beekman et al., 2000), but not in the larger ANSMHWB study (Goncalves et al., 2011) and none of these studies found significant associations with early childhood events.

We found that both recent and very distal adverse experiences having occurred during childhood more than 50 years before could predict late-life GAD, independently of other risk factors.

Shared environment, *e.g.* poverty, parental loss or separation, and low affective support in childhood were significantly associated with incident GAD. Negative parenting behavior and insecure attachment (Beesdo et al., 2010; Moffitt et al., 2007; Newman et al., 2013) as well as physical or sexual abuse (Chou, 2012; Cougle et al., 2010; Kessler et al., 2008) have already been shown to be associated with GAD in young and adult populations. However, previous studies failed to find a significant association between sexual and physical abuse and GAD in elderly subjects (Beekman et al., 2000). In our study, physical or sexual abuse was significantly associated with prevalent GAD in the model adjusted for age and sex, but this failed to be significant after multivariate adjustment and with incident GAD. This may be explained by a lack of power or under-reporting due to reluctance or denial to recall severe abuse, older adults being reported to recall less than younger adults negative relative to positive information (Charles et al., 2003).

Early adverse events could induce a persistent sensitization of CNS circuits which regulate stress and emotion. Exposure to stressful life events has been associated with CNS dysfunction and marked long-term changes in brain circuitry regulating stress reactivity involving the HPA axis (Faravelli et al., 2012). This may represent the underlying biological substrate of an increased vulnerability to subsequent stress as well as to the development of anxiety (Heim et al., 2001). A persistent activation of the HPA axis has been suggested from different observations, involving modifications in CRH and glucocorticoid receptors at the level of the hippocampus, limbic system, cortical areas, and neurotransmitter systems (Nemeroff, 1999). The long-lasting effects include structural and functional changes, notably reduced development of the hippocampus and amygdala, and abnormal fronto-temporal electrical activity (Teicher et al., 2002). A reduced negative feedback sensitivity of the HPA axis and sustained increase of cortisol level is reported in GAD (Henry, 1992; Schweizer et al., 1986; Tiller et al., 1988), and in the Esprit cohort, GAD has previously been shown to be associated with an increased secretion of diurnal cortisol under stress conditions (Chaudieu et al., 2008).

#### 4.4 Physical health

Previous epidemiological studies on physical health have mostly focused on global health condition (number of chronic diseases or somatic medications) and show significant associations in cross-sectional studies only (Goncalves et al., 2011; Lim et al., 2005; Muhsen et al., 2008; Schoevers et al., 2003), with therefore limited etiological relevance, chronic disorder possibly co-occurring or being a consequence of GAD (see Section 1, § 5.3). Disability was only evaluated in the AMSTEL study and was not significantly associated with incident late-life GAD (Schoevers et al., 2005). We also failed to find significant associations with mobility limitation as well as visual and hearing impairment which had not been examined before.

Specific chronic diseases (Carroll et al., 2011; Lim et al., 2005; Mackenzie et al., 2011; Muhsen et al., 2008) were only examined in four cross-sectional studies in adults, of which three reported a significant association between respiratory disorders and GAD (Carroll et al., 2011; Lim et al., 2005; Muhsen et al., 2008). Among a large range of vascular factors examined, only coronary artery disease in one study was associated with GAD (Lim et al., 2005). None of these chronic disorders were examined prospectively and/or in elderly people. In our study, we were able to examine a large range of common age-related chronic disorders, *e.g.* vascular, heart, respiratory, and thyroid disorders, cognitive impairment, as well as osteoporosis. Among the latter, cognitive impairment, respiratory disorders, arrhythmia and heart failure, and dyslipidemia were significant predictors of late-life GAD. Stress has a significant role in the etiology of these disorders they can themselves generate chronic stress, and dysfunction of the autonomic nervous system and HPA axis has been reported in GAD (Chaudieu et al., 2008; Hoehn-Saric et al., 2004).

#### 4.4.1 Cognitive impairment

Previous studies examining cognitive functioning were restricted to a global cognitive measure using MMSE and they failed to show significant associations with GAD (Beekman et al., 1998; Schoevers et al., 2005). In the AMSTEL study, cognitive impairment was significantly associated with prevalent but not incident GAD over 3 years (Schoevers et al., 2003; Schoevers et al., 2005). In the Esprit study, we were able to evaluate global cognitive function, but also specific cognitive domains, verbal fluency, visual memory, and cognitive skills related to visual scanning, psychomotor speed and executive function (TMT). Verbal fluency was the most significant and robust variable associated with incident GAD and also the only cognitive task associated with late onset GAD compared to early onset. Low performances on TMT and MMSE were associated with incident GAD using Cox models adjusted for sex, but not in multivariate models. A few case-control studies in the elderly support an association between GAD and deficits in cognitive control (*i.e.* inhibitory control in Stroop interference task, TMT, and verbal fluency) (Beaudreau et al., 2013; Butters et al., 2011; Caudle et al., 2007). In the Esprit study, an association between verbal fluency and cortisol levels has been reported previously (Beluche et al., 2010). Interestingly, in randomized controlled trial, SSRI treatment has been reported to improve both GAD symptomatology and neuropsychological functioning, associated with a decline in cortisol and cognitive improvement (Blay et al., 2012; Butters et al., 2011).

Emotional dysregulation and excessive worry seem to be an important cognitive dysfunction in GAD patients and fMRI studies suggest that this dysfunction may involve the cortical areas (PFC and ACC). In a recent review, researchers propose that older adults with GAD “fail to activate” the PFC involved in the down-regulation of negative emotions, during active thought suppression (Andreescu et al., 2012). Failure to activate the PFC appears specific to thought suppression, emotional Stroop, and simple motor tasks (Mohlman et al., 2009). Decreased ACC activation has been observed in younger adults during worry induction (Paulesu et al., 2010), but ACC activation in older adults with GAD is increased compared with late-life controls during a worry condition (Andreescu et al., 2011; Mohlman et al., 2009). The reason may be that young adults demonstrate increased functional efforts toward cognitive control of negative emotions, however, this process is disrupted in anxious older adults. A systematic review of fMRI studies also suggests that there is a hypo-activation of the cortical areas and a deficient cortex-amygdala functional connectivity in GAD patients (Mochcovitch et al., 2014), but the directionality of late-life anxiety and cognitive control remains to be ascertained (Beaudreau et al., 2013). Our study suggests that the presence of pre-existing cognitive deficits, notably on tasks depending on prefrontal processing could increase the risk of late-onset GAD.

#### 4.4.2 Adiposity and lipid levels

In response to stress, the autonomic nervous system as well as the HPA axis is centrally activated and their persistent dysregulation could lead to metabolic alterations, such as diabetes, altered lipid metabolism and adiposity (Chrousos, 2009; Pereira et al., 2013). In our study, diabetes and hypertension are not significantly associated with incident GAD, whereas lipid levels and WHR are differently associated with GAD. The fact that higher abdominal obesity (visceral fat) but not general body mass (peripheral fat) is a risk factor for incident GAD is however, consistent with an over-reactivity of the HPA axis. Peters et al. showed that exposure to chronic stress can result in two different phenotypes depending on genetic background (Peters et al., 2013). The A-phenotype accumulates visceral fat and does not habituate to chronic stress, whereas the B-phenotype, once exposed to stressful environment, habituates but needs to increase food intake to maintain brain energy metabolism, subsequently accumulating peripheral fat. The absence of habituation to stress in the A-phenotype has been linked to higher cortisol secretion during exposure to uncontrollable psychological stressors (Epel et al., 2000); an increased cortisol secretion under stress conditions was also previously reported in the participants with GAD in the Esprit study (Chaudieu et al., 2008).

High LDL-cholesterol levels but not vascular pathologies are associated with a decreased GAD incidence which may be consistent with neural mechanisms. Controversial findings are reported from cross-sectional studies with non-significant, positive, or negative associations with cholesterol (Troisi, 2009). A few small studies show an inverse association between anxiety and LDL-cholesterol in young adults (Conklin et al., 2008; Suarez, 1999). LDL-cholesterol is the major carrier of cholesterol, notably required for the regulation of cell membrane viscosity. Increase in serum LDL-cholesterol could be associated with increased brain cell membrane cholesterol, and changes in density and functioning of neurotransmitter transporters or receptors (Troisi, 2009). A negative association and interaction with 5-HTT for late-life depression has already been reported in the Esprit study (Ancelin et al., 2010) and experimental studies suggested that cholesterol may influence cholecystokinin and GABA receptors (Sooksawate et al., 2001).

#### **4.4.3 Respiratory disorders, heart failure, and arrhythmia**

Difficulty breathing and other autonomic arousal symptoms such as palpitations and accelerated heart rate are among the diagnostic criteria for GAD in the ICD-10 and hyperarousal was also one of the criteria for GAD diagnosis in the DSM-III-R, which was removed in the DSM-IV. We found that respiratory disorders as well as arrhythmia and heart failure were associated with an increased risk of incident but not prevalent GAD which most likely suggests that they are predictive factors of GAD. Reduced lung function, asthma, chronic obstructive pulmonary disease as well as breathlessness and frequent use of a bronchodilator have been found to be associated with prevalent GAD in adults (Carroll et al., 2011; Lavoie et al., 2011; Muhsen et al., 2008). Clinical studies on pharmacological or pulmonary rehabilitation treatments report significant declines in anxiety symptom severity linked to treatment (Brenes, 2003). There is also some evidence showing that respiratory disorders such as excessive and inadequate ventilation could result in the activation of the HPA axis (Abelson et al., 2010), also supporting our findings.

The respiratory system is also directly linked to the cardiovascular system via mechanical and neural pathways, and may play a role in moderating the impact of HPA axis on cardiovascular functioning (Courtney et al., 2011; Eckberg, 2003). An association between worry and a diminished heart rate stress response has been reported in young adults which suggests a possible suppression of adrenergic sympathetic stress response in GAD (Fisher et al., 2013). Respiratory dysfunction and heart rate variability could also reflect sympathetic and parasympathetic activity. High sympathetic activity coupled with low parasympathetic cardiac control has been shown to be associated with persistent hyperarousal symptoms in PTSD, which is also associated with respiratory abnormalities (Bleichert et al., 2007). The prospective design of our study suggests that respiratory problems and heart failure or arrhythmia could be risk factors for incident GAD.

#### **4.5 Mental health**

We found that current major depression, phobia, and past GAD were significantly associated with incident GAD. Hence, depression and phobia are likely to be risk factors in addition to being probable consequence of GAD. A personal history of depression/ anxiety symptomatology was also reported as a risk factor for GAD in the elderly AMSTEL cohort (Schoevers et al., 2005). The large longitudinal NESARC study found that several psychiatric disorders (*e.g.* unipolar and bipolar depression, phobia, panic disorder, and PTSD) could increase the risk of incident GAD in adults aged over 18 years (Grant et al., 2009) but when restricting the analysis to the elderly participants aged 60 years and over, only PTSD was a risk factor for late-life GAD (Chou et al., 2011). We did not examine PTSD and panic disorder but their low prevalence (less than 0.2%) suggests that there are unlikely to be significant risk factors in our population. We also found that a family history of mental disorders in parents could increase the risk of late-life GAD as reported in younger cohorts (Beesdo et

al., 2010; Kessler et al., 2008). This has not been examined before in the elderly and could reflect both early shared environment and genetic vulnerability to anxiety disorders.

## CHAPTER 5 – Genetic vulnerability related to adrenergic receptors

Of the eight genetic variants involved in the adrenergic function examined in our study, three were significantly associated with a 4-fold modified risk of GAD, namely *ADRA1A* *rs17426222* and *rs573514*, and *ADRB2* *rs1042713*, whereas no significant associations were found with the two *ADRA2* variants. The two *ADRA1A* SNPs are intronic and their functionality remains to be determined (Wang et al., 2007a) whereas the non-synonymous *ADRB2* variant can affect receptor down-regulation and agonist-induced *ADRB2* desensitization, as well as antagonist (beta-blockers) medication response and vascular reactivity (Litonjua et al., 2010; Taylor, 2007; Thakkestian et al., 2005). We also observed an association with a functional variant (*rs7903146*) of *TCF7L2* which can notably be activated by  $\beta 2$  adrenoreceptor agonists (Faisy et al., 2014) and can influence the transcription of a number of other *GPCRs* (Florez et al., 2006; Jin et al., 2008; Migliorini et al., 2015).

While no previous studies have investigated the role of *ADR* genetic variants in GAD, associations have been reported with vascular response to stress, cognitive function (Lyll et al., 2013), as well as specific psychiatric disorders, *i.e.* schizophrenia and PTSD (Clark et al., 2005; Liberzon et al., 2014). Variants in the promoter region of *ADRA1A* have been associated with schizophrenia in a Basque (Clark et al., 2005), but not in a Chinese population (Huang et al., 2008). A number of studies have also reported an association between *ADR* variants and obesity-related phenotypes in schizophrenia patients (Liu et al., 2010; Saliba et al., 2014). Liberzon et al. examined the associations between *ADR* variants and PTSD in trauma-exposed adults (Liberzon et al., 2014). None of the main effects survived correction for multiple testing, but significant interactions with childhood adversity on PTSD were detected (Liberzon et al., 2014). In our study, we found a significant association between *ADRB2* *rs1042713* and GAD, as well as an interaction with childhood adversity, with a lower risk of GAD specifically in the subjects who did not report low affective support during childhood.

GAD is often comorbid with other affective disorders and previous family studies suggest there may be overlapping genetic risk factors between GAD, PTSD, and panic disorder. In our sample, the prevalence of PTSD and panic disorder was low and we could not examine this possibility. Despite high phobia prevalence, no significant association was found between *ADR* variants and phobia further supporting our previous finding that GAD and phobia were etiologically different. Besides, the associations between *ADR* and GAD remained significant after further adjusting for depression which also suggests that depression was not a mediating factor. These findings are consistent with our finding that GAD was associated with some metabolic and somatic illnesses, which are related to the autonomic nervous system and the (nor)adrenergic system.

GAD is considered to be a disorder of emotional distress and phobia as a fear-based anxiety disorder (Durham et al., 2015). PTSD has elements of dysphoria as well as fear-based anxiety, and a recent study showed that PTSD dysphoria factor was uniquely related to GAD (Durham et al., 2015). Interestingly, significant interactions between *ADRB2* variants and either childhood adversity on PTSD (Liberzon et al., 2014) or maternal stress during pregnancy on autism in children (Cheslack-Postava et al., 2007) have been reported. Although speculative, this could suggest that *ADR* constitutes a vulnerability (or resilience) factor for the development of GAD following adversity, which may be shared by other stress-related disorders such as PTSD, possibly in its dysphoric dimension (Durham et al., 2015).

We also observed a significant interaction between *TCF7L2* *rs7903146* and history of adversity on GAD, the participants with the minor T allele reporting recent adverse events or low affective support during childhood being at a 3-5 fold higher risk of GAD compared with those who did not. *TCF7L2* plays an important role in the Wnt signaling pathway which can notably be activated by  $\beta 2$  adrenoreceptor agonists (Faisy et al., 2014) and can also influence the transcription of a number of other *GPCRs*. It is thereby implicated in a large variety of diseases and metabolic dysfunction (Florez et al., 2006; Jin et al., 2008; Migliorini et al., 2015). The direct mechanism linking this SNP with GAD is not known but it should be noted that it has also been associated with schizophrenia

(Hansen et al., 2011). Comorbidity and overlap between schizophrenia and anxiety have been reported as well as evidence for shared genes which could primarily have to do with reactivity and stress response (Ayalew et al., 2012). So far, few specific gene-environment interactions have been described for *TCF7L2* polymorphisms, mostly in relation with metabolic dysfunction but no previous studies have examined the role of upstream factors such as stressful environment.

This is the first study to explore the associations between several *ADR* variants and GAD in the elderly. The *ADRA1A* and *ADRB2* variants are strongly associated with late-onset GAD but not shared with depression or the other major anxiety disorder, phobia. Our data further support the critical role of the adrenergic nervous system in GAD, with possible modulating effects of *ADR* genes in response to recent or early stressful life events.

## CHAPTER 6 – Limitations and Strengths

### 6.1 Limitations

There are seven main limitations to be taken into account in these three studies:

1. Selection bias is potentially introduced as a result of the non-response of 24% of eligible inhabitants contacted for inclusion. This limits the generalizability of our findings (although not measurable, there is a probable healthy volunteer bias). In addition, institutionalized elderly people were excluded further limiting the generalizability of our findings to the general population of elderly subjects.
2. Subjects with missing data or lost to follow-up were excluded from the analyses, as well as those diagnosed with dementia at baseline to minimize recall bias. These subjects were older and with poorer health and thus more likely to be diagnosed with GAD which may have decreased the overall power of the study and led to an underestimation of the actual number of cases and of the strength of the associations found.
3. Data on life events especially during childhood were self-reported and retrospective and may have been subject to recall bias.
4. While potential over-adjustment may have occurred, it is also possible that there still remains residual confounding and those other unmeasured factors such as social environment and personality traits, may partly explain some associations reported here.
5. The risk factors for incident GAD were only considered at baseline and we did not consider the new physical disorders which may have occurred during the follow-up or the changes in risk factors over time.
6. Despite the large overall sample size, we were not able to analyze comorbidity with depression due to a low number of comorbid cases.
7. For the genetic study, the bias from population stratification also needs to be considered as French law prohibits collecting data related to ethnicity.

### 6.2 Strengths

Our study however, has six main strengths.

1. The subjects were randomly recruited from the fifteen electoral rolls of the Montpellier district. The sample size with over two thousand elderly people was large, which enabled adjustment for a large number of potential confounding factors and reduced the risk of random errors. It also allowed considering the interacting effects between some of the key covariates, such as *ADR* genes and stressful life events.
2. The data were derived from a longitudinal population-based study, with five follow-up examinations over 12 years, which increases the number of GAD events, and provides sufficient information for incidence estimates.
3. Differential diagnosis of specific anxiety disorders as well as depression was obtained by trained staff using a standardized psychiatric examination based on DSM-IV criteria and validated by a clinical panel, thus minimizing misclassification. Both past and current GAD were evaluated and the dates of first onset were registered to compare early and late onset GAD.
4. We have examined a great variety of factors including socio-demographic and lifestyle factors, lifetime traumatic events, medical history, neuropsychological assessment, as well as physical and mental disorders and comorbidity. Psychotropic and somatic medication use was ascertained from prescription and drug packages, thus minimizing exposure misclassification.
5. Some key variables like weight, height, waist, and hip were measured and not self-reported,

BMI and WHR were calculated, blood pressure was measured twice, and glycaemia and lipid levels were measured from blood samples after 12-h fasting.

6. The genotyping system used had a very low error rate, and we were able to control for accuracy through duplicate samples.

## **SECTION 6 – CONCLUSION AND PERSPECTIVES**

## CHAPTER 1– Main conclusions

GAD is a chronic disorder with a low rate of full remission and associated with increased disability and mortality in the elderly. It is frequently under-recognized and misdiagnosed and thus untreated in elderly people. However, as it is commonly assumed that late cases could principally represent the continuing course of early onset of illness and that high rates of anxiety are to be expected in the elderly due to increased vulnerability, there had been limited research into the clinical and etiological characteristics of late-life GAD.

The findings from this work provide novel information on GAD in a community-dwelling elderly population. GAD has relatively high prevalence and incidence rates in the elderly and is not an offside-effect of other psychopathologies frequent in the elderly, namely depression and phobia. Nearly two thirds of the GAD cases received no treatment. First episodes in late life are more common than previously believed and are related to specific factors consisting of environmental, intrinsic (stressful life events) as well as extrinsic factors, *i.e.* age-related chronic disorders, respiratory disorders, arrhythmia and heart failure, lipid levels, adiposity and cognitive impairment. Our findings also suggest a genetic vulnerability related to adrenergic receptors and support their possible modulating effect in response to stressful life events. This work thus suggests that GAD is a multifactorial psychiatric disorder showing longstanding vulnerability with possible biological origins involving stress systems.

## CHAPTER 2– Perspectives

These results are potentially interesting but require confirmation. Several suggestions are proposed for future studies:

1. Further studies are needed to replicate our data in large population samples in France and other countries, in order to validate our findings and explore the possible effect of culture and ethnicity.
2. Other potential risk factors which were not collected in the Esprit study should be examined such as social support and environment, network, and personality traits.
3. Larger samples would allow greater statistical power to confirm some marginal associations, examine potential gender differences, and explore in more detail other psychiatric disorders, notably panic disorder and PTSD. For these disorders, it would be interesting to examine the genetic vulnerability related to *ADR* genes and potential interactions with adverse environment and possible genetic risk factors overlapping with GAD.
4. *ADR* genes were examined for the first time in GAD in an elderly population, and it would be interesting to examine these genes in younger cohorts.
5. Further work in this area could involve investigating whether polymorphisms in other stress- or neurotransmitter- related genes are associated with late-life GAD. A DNA bank is available from over 1000 participants in the Esprit study and a large number of genes have already been genotyped. This could enable us to validate in elderly people findings of previous genetic association studies in adults, for instance on *5-HTT*, *BDNF*, or *MAO-A*. It would also allow us to examine for the first time for GAD, genes involved in the HPA axis and coding for glucocorticoid and mineralocorticoid receptors, CRH, CRH receptors, ACTH, and Arginine Vasopressin. Cortisol data are also available for more than 400 subjects which will permit us to study the functionality of some of these genetic variants. Interacting effects of adverse environment could also be evaluated.
6. In addition, MRI data of regional brain volumes are available for Esprit participants aged 85 years and less but they have not yet been analyzed in relation with GAD.

The identification of psychopathological, health- and stress- related risk factors as well as genetic vulnerability are essential for the early identification, and treatment of late life GAD, thus having potential major health and socio-economic consequences by decreasing comorbidity, disability, and mortality. Our findings provide an important lead for examining the pathogenesis of GAD involving the adrenergic stress system, as well as developing novel and specific preventative and intervention strategies.

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## INDEX OF TABLES

TABLE	PAGE
Table 1. Main large epidemiological community surveys of current and lifetime prevalence of common anxiety disorders	P 20
Table 2. DSM-5 (or DSM-IV-TR) diagnostic criteria for GAD	P 30
Table 3. Factors associated with prevalent GAD in large adult population-based studies	P 45
Table 4. Prospective studies having examined risk factors for incident GAD in general population.	P 48
Table 5. Candidate-gene studies on GAD	P 56
Table 6. Baseline characteristics of the 2259 participants in the Esprit study	P 78
Table 7. Summary of risk factors associated with prevalent and 12-year incident GAD in the Esprit study	P 89

## INDEX OF FIGURES

FIGURE	PAGE
Figure 1. Participant recruitment to the Esprit study	P 64
Figure 2. Number of Esprit participants over the 12-year follow-up.	P 65
Figure 3. General flow chart of the participants included in the analyses described in the thesis.	P 76