survival, neurite outgrowth and synaptic formation, and alterations of ALK functions may result in vulnerability to developing schizophrenia, which accords with the neurotrophic factor theory of schizophrenia (Thome et al, 1998; Durany and Thome, 2004). Indeed, alterations in other neurotrophic factors such as brain-derived neurotrophic factors (BDNF) and neurotrophin-3 have been implicated in schizophrenia (e.g., Durany et al, 2001; Nanko et al, 2003; Hattori et al, 2002).

A limitation in the present study might be that the obtained evidence for association was not very strong (p-values of <0.01 level in a single sample). Replication studies in independent samples are required. If our results are replicated, experiments elucidating the possible effects of the amino acid substitutions (Arg1491Lys and Glu1529Asp) on the ALK protein functions may serve to advance our understanding of the molecular mechanisms of schizophrenia and may provide clues to production of new treatment of the illness.

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Table 1 Genotype distributions and allele frequencies of the Glu1529Asp polymorphism of the ALK gene (rs1881421) in patients with schizophrenia and controls.

	Genotype distribution				Allele frequency		
	N	Glu/Glu	Glu/Asp	Asp/Asp	N	Glu	Asp
Patients	300	141 (47%)	128 (43%)	31 (10%)	600	410 (68%)	190 (32%)
Controls	308	171 (55%)	123 (40%)	14 (5%)	616	465 (75%)	151 (25%)

Discrepancy of performance among working memory related tasks in autism spectrum disorders was caused by task characteristics except working memory which could interfere with task execution.

Running Title: Discrepancy of performance among working memory related tasks in autism spectrum disorders.

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[ABSTRACT]
Aim: Working memory performance has been inconsistently reported in autism spectrum disorders (ASD). Several studies in ASD have found normal performance in digit span and poor performance in digit symbol task though these are closely related with working memory. We assumed that poor performance in digit symbol could be explained by confirmatory behavior due to the vague memory representation of number-symbol association. Therefore, we hypothesized that the performance of working memory task in which vagueness did not cause confirmatory behavior would be normal in ASD. For the purpose, Advanced Trail Making Test (ATMT) was introduced. We compared the performance of digit span, digit symbol and ATMT between ASD and normal control to test the hypothesis.
Methods: We performed digit span, digit symbol and ATMT to sixteen ASD subjects and twenty-eight IQ, age and sex matched control subjects. The scores of these tasks were compared.

Results: A significantly lower score of ASD was shown only in digit symbol compared with control subjects. There were no significant difference in digit span and working memory estimated by ATMT.
Conclusions: Discrepancy of scores among working memory related tasks was demonstrated in ASD. Poor digit symbol performance, normal digit span and normal working memory in ATMT implied that ASD subjects were intact in working memory itself, and that superficial working memory dysfunction could be observed due to confirmatory behavior in digit symbol. Therefore, to evaluate working memory in ASD, tasks which could stimulate psychopathology specific to ASD should be avoided.

Key words: autism spectrum disorders, working memory, vagueness, digit symbol, digit span

INTRODUCTION

Working memory refers to a cognitive function that provides concurrent temporary storage and manipulation of the information necessary for complex cognitive tasks.1 For the past three decades, numerous studies have reported executive dysfunction in autism spectrum disorders (ASD).2-5 Working memory is generally considered one of executive functions,2-4,6,7 but working memory performance in ASD has been inconsistently reported until now. Some studies found deficiency in working memory,7-14 though others reported normal performance in ASD.13-19 This inconsistency among the studies might be attributed to the task characteristics. Most of the working memory studies in ASD utilized original tasks not standardized, hence the cognitive factors necessary for task performance except working memory were different among studies.

Even in a standardized cognitive battery like Wechsler Intelligence Scale,20 the situation regarding working memory in ASD was quite similar. In the subtests of this battery, digit span and digit symbol are closely related with working memory. In digit span test, subject must memorize and repeat strings of digits in forward and backward order, and in digit symbol test, memorization of number-symbol association could enhance task performance. In ASD, several studies have found certain profiles of these tests, i.e., normal score in digit span and poor score in digit symbol.7, 21,22 Thus, even a standardized tool like Wechsler Intelligence Scale showed discrepancy between the scores of subtests related with working memory. Besides, there have never been any researches discussing the cause of deterioration of digit symbol in ASD so far.

To interpret the deterioration of digit symbol in ASD, we assumed that the poor digit symbol performance might be attributed not to disturbance of working memory itself but to deficit in acting on the basis of retained vague information because it was considered that vagueness of working memory representation might cause repetitive confirmatory behavior which compelled ASD subjects to check the correct number-symbol association and which would result in the delay of task performance.

According to this consideration, we hypothesized that working memory task in which

vague memory representation did not provoke confirmatory behavior would yield normal performance in ASD. For the purpose, a novel task named Advanced Trail Making Test (ATMT) was introduced. The ATMT, developed by Kajimoto et al.23 is a computerized version of Trail Making Test24,25. This task is carried out using visuomotor coordination, visual scanning like digit symbol test and is able to estimate quantitatively working memory, which could not be affected by the vague memory representation because the vagueness of memory on the positions of subsequent numbers provoke no confirmatory behavior. Therefore we adopted ATMT as a control task for digit symbol. These two tasks were different in the point of whether vagueness of memory could result in confirmatory behavior or not.

In the present study, we compared the performance of digit span, digit symbol and ATMT in ASD with those in normal control to test the hypothesis that discrepancy of working memory task performance in ASD could be explained by the task characteristics except working memory.

Susceptibility genes for schizophrenia

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Key Words: schizophrenia, susceptibility gene, dysbindin, neuregulin-1, DISC1, COMT, G72, RGS4, Akt

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Abstract

It is well known that genetic factors contribute to the susceptibility for schizophrenia. Recent advance of the molecular genetics of schizophrenia strongly suggests several susceptibility genes, e.g. dysbindin, neuregulin-1, DISC1, COMT, G72, RGS4 and Akt1. We discuss the evidence and biology of these genes. As glutamate transmission is especially implicated in these genes, neurobiological basis of schizophrenia might be elucidated by investigation of functional interactions between susceptibility genes for schizophrenia and glutamatergic system.

Introduction

Schizophrenia is a major mental disorder that is one of the world's top ten causes of long-term disability. This disease is characterized by psychosis and profound disturbances of cognition, emotion and social functioning. It affects approximately 1%

of the general population across different countries and cultural group worldwide. The fact that schizophrenia has a genetic component has long been established with high heritability estimates of 80%1, 2. As the genetic transmission does not appear to follow simple mendelian single-gene inheritance pattern, this disease is a complex genetic disorder, like other common diseases. Many years of much effort has devoted to identify susceptibility genes for schizophrenia. As a result, genome wide linkage studies suggested several positive linkage regions such as 1q, 5q, 6p, 6q, 8p, 10p, 13q, 22q3, 4. A recent meta-analysis showed evidence for linkage at 8p, 13q and 22q5, and another meta-analysis at 1q, 2p, 2q, 3p, 5q, 6p, 8p, 11q, 14q, 20p, 22q6. The chromosomal abnormalities in schizophrenia have also added the evidence for susceptibility loci at 1q427, 8 and 22q119-11. A number of susceptibility genes for schizophrenia, including dysbindin, neuregulin-1, DISC1, COMT, and G72 and RGS4, have recently been identified in these loci12-17. The evidence for several genes becomes stronger now, as replication studies have achieved greater consistency than in the past18. Here we discuss the genetic evidence and biology of these susceptibility genes.

Dysbindin

A recent study implicated a gene on chromosome 6p, dysbindin (DTNBP1: dystrobrevin binding protein 1), as a susceptibility locus in the Irish pedigrees12. Since then, a significant association between schizophrenia and genetic variation in dysbindin has been reported in various populations from Ireland, Wales, Germany/Hungary/Israel, Sweden, Bulgaria, Unite States, China, and Japan19-27. One study, which initially failed to replicate a positive association based on SNPs in an Irish population, became subsequently positive using a haplotype strategy28. Thus, genetic evidence for association with schizophrenia is quite strong. Talbot et al.29 found that dysbindin protein levels were reduced in the hippocampal formation of patients with schizophrenia. This presynaptic reduction was observed especially in the inner molecular layer of the dentate gyrus. The expression levels of dysbindin mRNA and protein were also reduced in the prefrontal cortex in schizophrenic brains30, 31.

Dysbindin is originally found as a binding partner of alpha- and beta-dystrobrevins, which are causative genes of Duchenne muscular dystrophy 32. Dystrobrevins are parts of the dystrophin-associated protein complex which plays important roles in normal function of muscle33. Cognitive impairments are commonly found in patients with Duchenne muscular dystrophy and it is thought to be due to an abnormality in the neuronal membrane that is caused by lack of dystrophin34. A model mouse of Hermansky-Pudlak syndrome, sandy mouse, is caused by a nonsense mutation in the dysbindin gene35. This disease is characterized by oculocutaneous albinism, prolonged bleeding and pulmonary fibrosis due to abnormal vesicle trafficking to lysosomes and related organelles36. Dysbindin is a component of the biogenesis of lysosome-related organelles complex (BLOC-1) and reduced expression of other proteins in this complex has been found in the sandy mouse35. Altered expression of dysbindin in schizophrenic brain might affect the expression of BLOC-1, which could result in the abnormal protein trafficking in schizophrenia. Although several findings of function of dysbindin have been reported, little is known about the functions in neurons.

Numakawa et al.20 have recently shown that dysbindin might influence exocytotic glutamate release via up-regulation of the molecules in pre-synaptic machinery. They also reported that dysbindin promotes neuronal viability through PI3K-Akt signaling20. Impairments of these functions of dysbindin could play an important role in the pathogenesis of schizophrenia.

Neuregulin-1

Neuregulin-1 (NRG-1), which maps to the 8p locus, has been shown as a susceptibility gene for schizophrenia by a combination of linkage and association analysis13. Additional evidence for association with schizophrenia has been reported by ten independent groups37-46, whereas three studies failed to replicate it47-49. Notably, the majority of positive markers are located at the 5' region of this gene, which is close to the first exons encoding type IV and type II of NRG-1. Quite strong evidence for association with schizophrenia is suggested. Hashimoto et al.50 studied NRG-1 mRNA expression in dorsolateral prefrontal cortex (DLPFC) and found increased type I NRG1 mRNA in schizophrenia. The elevation of type I expression was present relative to three house keeping genes and to other NRG-1 isoforms (type II and type III). However, type I NRG1 mRNA expression levels correlated with neuroleptic doses in patients with schizophrenia, thus it is unclear this finding reflected a neuroleptic effect or disease severity. It is notable that Law et al.51 replicated the increased mRNA expression of type I NRG-1 in a much larger and separate sample in hippocampus and did not find any correlation between medication and NRG1 mRNA.

NRG-1 is one of the neuregulin family of proteins, which have a broad range of bioactivities in the central nervous system and contain an epidermal growth factor (EGF)-like motif that activates membrane-associated tyrosine kinase related to ErbB receptors52. NRG-1 regulates the expression and plasticity of N-methyl-d-aspartate (NMDA) receptors, of the β2 subunit of the γ-amino butyric acid (GABA) receptor and of nicotinic acetylcholine receptor subtypes including α5, α7 and β4 subunits53-56. A gene targeting approach for NRG-1-ErbB signaling revealed a behavioral phenotype in mice that overlaps with certain animal models for schizophrenia. For example, NRG-1 and ErbB4 mutant mice exhibit elevated activity levels in an open field, which was reversed by clozapine, and abnormal sensorimotor gating measured by prepulse inhibition of the startle reflex13, 57. The NRG-1 gene generates multiple alternative splicing variants, classified into three primary isoform groups (types I: heregulin / acetylcholine receptor inducing activity / neu differentiation factor, II: glial growth factor, III: sensory and motor neuron-derived factor)58, and recently additional 5' exon containing transcripts (types IV, V, VI) have been found in human brain59. These NRG-1 isoforms play multiple and distinct functions in neuronal development, which may be relevant to neurodevelopmental abnormalities in schizophrenia.

DISC1

The Disrupted in Schizophrenia 1 (DISC1) gene has initially been identified at the breakpoint of a balanced translocation (1;11)(q42.1;q14.3), which segregates with schizophrenia and related psychiatric disorders in a large Scottish family7, 14. Five

studies reported a significant association between schizophrenia and genetic variation in the DISC1 gene[60-64] and we also found such an association (Hashimoto et al., unpublished). However, two studies failed to find the association[65, 66]. There is evidence for association with bipolar disorder[62, 64] and with major depression (Hashimoto et al., unpublished). A frameshift mutation of the DISC1 gene has been found in an American family with schizophrenia and schizoaffective disorder[67]. These findings suggest that DISC1 may give a susceptibility to mood disorders as well as schizophrenia. The function of DISC1 is still unclear, however, increasing evidence suggests a role in cytoskeletal organization, as DISC1 interacting proteins are associated with the components of microtubule and actin[68-71]. DISC1 is likely to be involved in the neurite extension[68, 70] and mitochondrial and nuclear related functions have also been suggested[69, 72-74].

COMT

Catechol O-methyltransferase (COMT) is a susceptibility gene for schizophrenia, which maps to 22q11 implicated in two meta-analyses of linkage studies[5, 6]. Hemideletion of this region produces velo-cardio-facial syndrome (VCFS), a condition associated with increased risk of schizophrenia-like psychoses[75]. COMT is a key enzyme in the elimination of dopamine in the prefrontal cortex. A functional polymorphism of the COMT gene, Val158Met, affects prefrontal function, and the high-activity val allele has been reported to be a genetic risk factor for schizophrenia in at least eight studies[18]. Among the susceptibility genes for schizophrenia, only COMT has evidence for the association with functional polymorphism. As COMT val allele is associated with prefrontal abnormalities, COMT is linked more strongly with cognitive intermediate phenotypes, e.g. executive function, cortical processing and P300 evoked EEG response[15, 76, 77]. The mRNA expression levels of COMT in schizophrenia has been studied in DLPFC and they show only minor alterations[78, 79]. Many negative results have also been reported and recent meta-analysis was inconclusive[80], however, it is likely that the COMT Val158Met polymorphism is a part of the complex risk architecture of schizophrenia[18].

G72

G72 was cloned from a 5 MB gene desert in the 13q linkage region[16]. Biochemical study revealed that G72 protein activated D-amino acid oxidase (DAAO), which was involved in the metabolism of D-serine, an agonist at the glycine modulately site of the NMDA receptor[16]. Chumakov et al. also reported that DAAO was associated with schizophrenia[16]. Subsequently, five studies suggested a significant association between schizophrenia and G72[81-85], whereas one study did not support the association[86]. As the association with child-onset schizophrenia and with bipolar disorder has also been reported[83, 87, 88], this gene is likely to be a susceptibility gene for psychosis. The increased expression of G72 mRNA was observed in DLPFC of postmortem brain in patients with schizophrenia, which is consistent with glutamatergic theory of schizophrenia.

RGS4

Regulator of G-protein signaling 4 (RGS4) has been discovered to be decreased in the prefrontal cortex of patients with schizophrenia using cDNA microarrays17. RGS4 maps to 1q, one of the suggestive linkage regions3, 4, 6. Five reports suggested the association with schizophrenia89-93, while two studies failed to replicate it94, 95. RGS4 deficient mice showed normal behavior including intact prepulse inhibition, except subtle sensorimortor abnormality96. RGS4 accelerates the GTPase activities of G protein alpha-subunits and negatively modulates G protein–mediated signaling via dopamine, metabotropic glutamate, and muscarinic receptors. The evidence for genetic association between schizophrenia and RGS4 is suggestive.

Akt1

Akt1 (protein kinase B) is implicated as a susceptibility gene for schizophrenia using a combination of experiments97. Emamian et al.97 reported reduced expression of Akt1 protein in lymphocytes and postmortem brain tissue of patients with schizophrenia and genetic association between Akt1 and schizophrenia. They also demonstrated higher sensitivity to amphetamine-induced PPI disruption in Akt1 knockout mouse97. Subsequent two studies supported the evidence for association of variants in the Akt1 gene with schizophrenia98, 99, whereas one study failed to replicate it100. Akt has emerged as the focal point for many signal transduction pathways, regulating multiple cellular processes such as glucose metabolism, transcription, apoptosis, cell proliferation, angiogenesis, and cell motility101. In the central nervous system, the PI3K-Akt signaling pathway plays a critical role in mediating survival signals102, 103. PI3-kinase-Akt signaling is also involved in the survival promoting effect of dysbindin20. Despite weak linkage evidence of Akt1 (14q) and small number of positive association studies, biological evidence strengthens the candidacy of Akt1 as a susceptibility gene for schizophrenia.

Conclusion

Several studies have replicated genetic association between polymorphisms in dysbindin, neuregulin-1, DISC1, COMT, G72, RGS4, and Akt1 and schizophrenia. However, no causative polymorphism has not been described in schizophrenia, except for the val allele in the COMT gene. Discovery of the causative mutation is the next step of this field. As biological evidence of these genes accumulates in the glutamate transmission, further investigations of functional connectivity among these susceptibility genes and glutamatergic system should be conducted.

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The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia

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The catechol-O-methyl transferase (COMT) gene is considered to be a promising schizophrenia susceptibility gene. A common functional polymorphism (Val158Met) in the COMT gene affects dopamine regulation in the prefrontal cortex (PFC). Recent studies suggest that this polymorphism contributes to poor prefrontal functions, particularly working memory, in both normal individuals and patients with schizophrenia. However, possible morphological changes underlying such functional impairments remain to be clarified. The aim of this study was to examine whether the Val158Met polymorphism of the COMT gene has an impact on brain morphology in normal individuals and patients with schizophrenia. The Val158Met COMT genotype was obtained for 76 healthy controls and 47 schizophrenics. The diagnostic effects, the effects of COMT genotype and the genotype-diagnosis interaction on brain morphology were evaluated by using a voxel-by-voxel statistical analysis for high-resolution MRI, a tensor-based morphometry. Patients with schizophrenia demonstrated a significant reduction of volumes in the limbic and paralimbic systems, neocortical areas and the subcortical regions. Individuals homozygous for the Val-COMT allele demonstrated significant reduction of volumes in the left anterior cingulate cortex (ACC) and the right middle temporal gyrus (MTG) compared to Met-COMT carriers. Significant genotype-diagnosis interaction effects on brain morphology were noted in the left ACC, the left parahippocampal gyrus and the left amygdala-uncus. No significant genotype effects or genotype-diagnosis interaction effects on morphology in the dorsolateral PFC (DLPFC) were found. In the control group, no significant genotype effects on brain morphology were found. Schizophrenics homozygous for the Val-COMT showed a significant reduction of volumes in the bilateral ACC, left amygdala-uncus, right MTG and left thalamus compared to Met-COMT schizophrenics. Our findings suggest that the Val158Met polymorphism of the COMT gene might contribute to morphological abnormalities in schizophrenia.

Keywords: schizophrenia; polymorphism; COMT; ACC; DLPFC

Abbreviations: ACC = anterior cingulate cortex; COMT = catechol-O-methyl transferase; DLPFC = dorsolateral prefrontal cortex; FDR = false discovery rate; IQ = intelligence quotient; JART = Japanese version of National Adult Reading Test; ROI = region of interest; SPM = statistical parametric mapping; TBM = tensor-based morphometry

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Introduction

Schizophrenia is a severe neuropsychiatric disorder with deficits of multiple domains of cognitive functions, volition and emotion. Family and twin studies have provided cumulative evidence for a genetic basis of schizophrenia (Kendler, 1983; McGue *et al.*, 1983; Sullivan *et al.*, 2003); however, identification of the underlying susceptibility loci has been limited. Collective data have suggested that the aetiology of schizophrenia involves the interplay of complex polygenic influences and environmental risk factors operating on brain maturational processes (Harrison *et al.*, 2005).

In vivo neuroimaging studies have demonstrated that brain abnormalities should play an important role in the pathophysiology of schizophrenia. Structural MRI studies have demonstrated relatively consistent brain abnormalities in patients with schizophrenia, such as enlargement of the ventricular system and regional volume decrease in the temporal lobe structures (Gaser *et al.*, 2001; Okubo *et al.*, 2001; Shenton *et al.*, 2001; Davidson and Heinrichs, 2003). Studies with schizophrenics and their healthy siblings demonstrate that even healthy siblings share some of morphological abnormalities observed in schizophrenia (Steel *et al.*, 2002; Gogtay *et al.*, 2003). A recent morphological MR study revealed that a common polymorphism of the brain-derived neurotrophic factor, one of the well-known schizophrenia susceptibility genes, affected the anatomy of the hippocampus and prefrontal cortex (PFC) in healthy individuals (Pezawas *et al.*, 2004). Furthermore, some studies have suggested that environmental factors interact with genetic factors (Cannon *et al.*, 1993; Nelson *et al.*, 2004). For example, obstetric complications are well known non-genetic risk factors of schizophrenia. However, a previous study suggested that obstetric complications might induce brain morphological abnormalities in schizophrenics and their siblings, but not in comparison with subjects at low genetic risk for schizophrenia (Cannon *et al.*, 1993). These facts suggest that genetic factors should have considerable impact on brain morphology in patients with schizophrenia.

Catechol-O-methyl transferase (COMT) is a promising schizophrenia susceptibility gene because of its role in monoamine metabolism (Goldberg *et al.*, 2003; Stefanis *et al.*, 2004; Harrison *et al.*, 2005). A common single nucleotide polymorphism (SNP) of the COMT gene producing an amino acid substitution of methionine (met) to valine (val) at position 108/158 (Val158Met) affects dopamine regulation in the PFC (Palmatier *et al.*, 1999). This polymorphism impacts on the stability of the enzyme, such that the Val-COMT allele has significantly lower enzyme activity than the Met-COMT allele (Weinberger *et al.*, 2001; Chen *et al.*, 2004). Several studies have revealed that the Val-COMT allele is associated with poorer performances, compared to the Met-COMT allele, in cognitive tasks of frontal function such as the Wisconsin Card Sorting Test (WCST) and N-back task (Egan *et al.*, 2001; Weinberger *et al.*, 2001; Goldberg *et al.*, 2003). The underlying mechanism of such behavioural differences may be related to lower prefrontal dopamine levels arising from higher dopamine catabolism mediated by the Val-COMT allele (Chen *et al.*, 2004; Tunbridge *et al.*, 2004).

The results of studies on the association between the Val158Met polymorphism and schizophrenia have, however, been controversial (Daniels *et al.*, 1996; Kunugi *et al.*, 1997; Ohmori *et al.*, 1998; Norton *et al.*, 2002; Galderisi *et al.*, 2005; Ho *et al.*, 2005). The result of a meta-analysis was even more inconclusive (Fan *et al.*, 2005). Such inconsistency was also found in associations between frontal functions and the Val158Met polymorphism (Egan *et al.*, 2001; Weinberger *et al.*, 2001; Goldberg *et al.*, 2003; Ho *et al.*, 2005). The possible morphological changes due to the COMT gene might be present and play a role in susceptibility to schizophrenia and in giving rise to impaired frontal functions. However, morphological changes underlying functional impairments remain to be clarified.

A recent advancement of methods for MR volumetry, such as voxel-based morphometry and deformation-based morphometry [or tensor-based morphometry (TBM)], allows us to explore and analyse brain structures of schizophrenics (Wright *et al.*, 1995; Gaser *et al.*, 2001). Using TBM techniques, we investigated the association between the Val158-Met polymorphism of the COMT gene and brain morphology in normal individuals and patients with schizophrenia. The aim of this study was to clarify whether there are significant genotype and/or genotype-disease interaction effects on brain morphology.

Methods

Subjects

Seventy-six healthy subjects and forty-seven patients with schizophrenia participated in the study. All the subjects were biologically unrelated Japanese. Written informed consent was obtained from all the subjects in accordance with ethical guidelines set by a local ethical committee. All normal subjects were screened using a questionnaire on medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the CNS, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarctions detected by T_2-weighted MRI, hypertension, chronic lung