INTRODUCTION

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Bronchial Asthma

Definition

Bronchial asthma is the common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm.[1] Symptoms include wheezing, coughing, chest tightness, and shortness of breath.[2] Asthma is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV1), and peak expiratory flow rate (PEFR).[3] Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic), based on whether symptoms are precipitated by allergens (atopic) or not (non-atopic).[4]

It is thought to be caused by a combination of genetic and environmental factors. [5] Its diagnosis is usually made based on the pattern of symptoms and/or response to therapy over time. [6]

Epidemiology

As of 2009, 300 million people worldwide were affected by asthma leading to approximately 250,000 deaths per year. [7-9]

It is estimated that asthma has 7-10% prevalence worldwide.[10] As of 1998, there was a great disparity in the prevalence of asthma across the world, with a trend toward more developed and westernized countries having higher rates of asthma,[11] with as high as a 20 to 60-fold difference. Westernization however does not explain the entire difference in asthma prevalence between countries, and the disparities may also be affected by differences in genetic, social and environmental risk factors.[12] Mortality however is most common in low to middle income countries,[13] while symptoms were most prevalent (as much as 20%) in the United Kingdom, Australia, New Zealand, and Republic of Ireland; they were lowest (as low as 2–3%) in Eastern Europe, Indonesia, Greece, Uzbekistan, India, and Ethiopia.[11]

Asthma affects approximately 7% of the population of the United States [14] and 5% of people in the United Kingdom. [15] Asthma causes 4,210 deaths per year in the United States.[10,15] More boys have asthma than girls, but more women have it than men.[16] In England, an estimated 261,400 people were newly diagnosed with asthma in 2005; 5.7 million people had an asthma diagnosis and were prescribed 32.6 million asthma-related prescriptions.[17]

Classification

Asthma is defined by the Global Initiative for Asthma as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable

airflow obstruction within the lung that is often reversible either spontaneously or with treatment". [18]

Table (1): Clinical classification of severity of bronchial asthma: [3]

Severity in patients ≥12 years of age[19]	**Symptom frequency**	**Night time symptoms**	**%FEV_1 of predicted**	**FEV_1 Variability**	**Use of short-acting $beta_2$ agonist for symptom control (not for prevention of EIB)**
Intermittent	≤2 per week	≤2 per month	≥80%	<20%	≤2 days per week
Mild persistent	>2 per week but not daily	3–4 per month	≥80%	20–30%	>2 days/week but not daily
Moderate persistent	Daily	>1 per week but not nightly	60–80%	>30%	Daily
Severe persistent	Throughout the day	Frequent (often7×/week)	<60%	>30%	Several times per day

Although asthma is a chronic obstructive condition, it is not considered as a part of chronic obstructive pulmonary disease as this term refers specifically to combinations of disease that are irreversible such as bronchiectasis, chronic bronchitis, and emphysema.[19]Unlike these diseases, the airway obstruction in asthma is usually reversible; however, if left untreated, the chronic inflammation of the lungs during asthma can become irreversible obstruction due to airway remodeling.[20]In contrast to emphysema, asthma affects the bronchi, not the alveoli.[21]

1) Brittle asthma

Brittle asthma is a term used to describe two types of asthma, distinguishable by recurrent, severe attacks. [2]Type 1 brittle asthma refers to disease with wide peak flow variability, despite intense medication. Type 2 brittle asthma describes background well-controlled asthma, with sudden severe exacerbations. [2]

2) Asthma attack

An acute asthma exacerbation is commonly referred to as an *asthma attack*. The classical symptoms are shortness of breath, wheezing, and chest tightness.[2] While these are the primary symptoms of asthma, [22] some people present primarily with coughing, and in severe cases, air motion may be significantly impaired such that no wheezing is heard.[2]

3) Status asthmaticus

Status asthmaticus is an acute exacerbation of asthma that does not respond to standard treatments of bronchodilators and steroids. Nonselective beta blockers (such as Timolol) have caused fatal status asthmaticus. [23]

4) Exercise-induced

A diagnosis of asthma is common among top athletes. There appears to be a relatively high incidence of asthma in sports such as cycling, mountain biking, and long-distance running, and a relatively lower incidence in weightlifting and diving. It is unclear how much of these disparities are from the effects of training in the sport. [24, 25]

Exercise-induced asthma can be treated with the use of a short-acting beta2 agonist.[19]

5) Occupational

Asthma as a result of (or worsened by) workplace exposures is a commonly reported occupational respiratory disease. Most cases were associated with the manufacturing (41.4%) and services (34.2%) industries.[26]Animal proteins, enzymes, flour, natural rubber latex, and certain reactive chemicals are commonly associated with work-related asthma. When recognized, these hazards can be mitigated, dropping the risk of disease. [27]

Causes of Bronchial asthma

Asthma is caused by environmental and genetic factors. [5] These factors influence how severe asthma is and how well it responds to medication. [28] The interaction is complex and not fully understood. [29]

1) Environmental

Many environmental risk factors have been associated with asthma development and morbidity in children. Recent studies show a relationship between exposure to air pollutants (e.g. from traffic) and childhood asthma. [30]

Viral respiratory infections are not only one of the leading triggers of an exacerbation but may increase one's risk of developing asthma especially in young children. [19]

Respiratory infections such as rhinovirus, *Chlamydia pneumoniae* and *Bordetella pertussis* are correlated with asthma exacerbations. [31]

Psychological stress has long been suspected of being an asthma trigger, but only in recent decades has convincing scientific evidence substantiated this hypothesis. Rather than stress directly causing the asthma symptoms, it is thought that stress modulates the immune system to increase the magnitude of the airway inflammatory response to allergens and irritants. [12, 32]

Beta blocker medications such as metoprolol may trigger asthma in those who are susceptible. [33]

*** Tobacco**

Maternal tobacco smoking during pregnancy and after delivery is associated with a greater risk of asthma-like symptoms, wheezing, and respiratory infections during childhood.

*** Hygiene hypothesis**

Antibiotic use early in life has been linked to development of asthma[34]in several examples; it is thought that antibiotics make children who are predisposed to atopic immune responses susceptible to development of asthma because they modify gut flora, and thus the immune system (as described by the hygiene hypothesis).[35]

***Volatile organic compounds (VOCs)**

Exposure to VOCs is associated with an increase in the interlukin-4 (IL-4) producing T-helper 2 (Th2) cells and a reduction in interferon- γ (IFN-γ) producing T-helper 1(Th1) cells. Thus the mechanism of action of VOC exposure may be allergic sensitization mediated by a Th2 cell phenotype. [36]

2) Genetic

Over 100 genes have been associated with asthma in at least one genetic association study. [37] However, such studies must be repeated to ensure the findings are not due to chance.

3) Exacerbation

Home factors that can lead to exacerbation include dust, house mites, animal dander (especially cat and dog hair), cockroach allergens and molds at any given home.[38] Perfumes are a common cause of acute attacks in females and children. Both virus and bacterial infections of the upper respiratory tract infection can worsen asthma. [38]

4) Socioeconomic factors

The incidence of asthma is highest among low-income populations worldwide. Asthma deaths are most common in low and middle income countries, [39] and in the Western world, it is found in those low-income neighborhoods whose populations consist of large percentages of ethnic minorities. [40] Additionally, asthma has been strongly associated with the presence of cockroaches in living quarters; these insects are more likely to be found in those same neighborhoods. [41]

Signs and symptoms

Common symptoms of asthma include wheezing, shortness of breath, chest tightness and coughing, and use of accessory muscle. Symptoms are often worse at night or in the early morning, or in response to exercise or cold air. [2] Some people with asthma only rarely experience symptoms, usually in response to triggers, whereas other may have marked persistent airflow obstruction.

***Gastro-esophageal reflux disease (GERD)**

Gastro-esophageal reflux disease coexists with asthma in 80% of people with asthma, with similar symptoms. Various theories say that asthma could facilitate GERD and/or vice versa. The first case could be due to the effect of change in thoracic pressures, use of antiasthma drugs, could facilitate the passage of the gastric content back into the esophagus by increasing abdominal pressure or decreasing the lower esophageal sphincter. The second by promoting bronchoconstriction and irritation by chronic acid aspiration, vaguely mediated reflexes and others factors that increase bronchial responsiveness and irritation. (42)

*** Sleep disorders**

Due to altered anatomy of the respiratory tract: increased upper airway adipose deposition, altered pharynx skeletal morphology, and extension of the pharyngeal airway; leading to upper airway collapse. (43)

Table (2): Diagnosis of bronchial asthma:

Severity of acute asthma exacerbations(2)		
Near-fatal asthma	High $PaCO_2$ and/or requiring mechanical ventilation	
Life threatening asthma	Any one of the following in a person with severe asthma:-	
	Clinical signs	**Measurements**
	Altered level of consciousness	Peak flow< 33%
	Exhaustion	Oxygen saturation< 92%
	Arrhythmia	PaO_2< 8 kPa
	Low blood pressure	"Normal" $PaCO_2$
	Cyanosis	
	Silent chest	
	Poor respiratory effort	
Acute severe asthma	Any one of:-	
	Peak flow 33–50%	
	Respiratory rate ≥ 25 breaths per minute	
	Heart rate ≥ 110 beats per minute	
	Unable to complete sentences in one breath	
Moderate asthma exacerbation	Worsening symptoms	
	Peak flow 50–80% best or predicted	
	No features of acute severe asthma	

Differential diagnosis

Differential diagnoses include: [1]

- Chronic obstructive pulmonary disease(COPD) (e.g., chronic bronchitis or emphysema)
- Congestive heart failure
- Pulmonary embolism
- Mechanical obstruction of the airways (benign and malignant tumors)
- Pulmonary infiltration with eosinophilia
- Cough secondary to drugs (e.g., angiotensin-converting enzyme (ACE) inhibitors)
- Vocal cord dysfunction

Prognosis

The prognosis for asthma is good, especially for children with mild disease. [44] Of asthma diagnosed during childhood, 54% of cases will no longer carry the diagnosis after a decade. The extent of permanent lung damage in people with asthma is unclear. Airway remodeling is observed, but it is unknown whether these represent harmful or beneficial changes. [45] Although conclusions from studies are mixed, most studies show that early treatment with glucocorticoids prevents or ameliorates decline in lung function as measured by several parameters. [46] For those who continue to suffer from mild symptoms, corticosteroids can help most to live their lives with few disabilities. It is more likely to consider immediate medication of inhaled corticosteroids as soon as asthma attacks occur. According to studies conducted, patients with relatively mild asthma who have received inhaled corticosteroids within 12 months of their first asthma symptoms achieved good functional control of asthma after 10 years of individualized therapy as compared to patients who received this medication after 2 years (or more) from their first attacks. Though they (delayed) also had good functional control of asthma, they were observed to exhibit slightly less optimal disease control and more signs of airway inflammation.

9) Prevention

The evidence for the effectiveness of measures to prevent the development of asthma is weak.[1] Ones which show some promise include limiting smoke exposure both in utero and after delivery, breastfeeding, increased exposure to respiratory infection per the hygiene hypothesis (such as in those who attend daycare or are from large families).[1]

Fibulins

The fibulins are a family of proteins that are associated with basement membranes and elastic extracellular matrix fibers.

In little more than a decade since the discovery of the first fibulin, a six-member family of extracellular-matrix (ECM) proteins have emerged [47] (Table 3; Fig.I).

The fibulins are minimally defined as having a series of epidermal growth factor (EGF)-like modules, followed by a carboxy-terminal fibulin-type module.

It is evident that the fibulins are an ancient family of proteins, which are highly conserved in species as evolutionarily distant as worms and humans. Fibulins have a diverse array of protein ligands. [48]

As a consequence of these widespread interactions, fibulins are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular ECM structures, such as elastic fibers and basement membranes. Indeed, the family name originates from the Latin word *fibula*, which means clasp or buckle.

Types of fibulins:

Table (3): Fibulin family nomenclature

Name	Synonymous name	Gene symbol	Human chromosome location
Fibulin-1 [49,50]	BM90	FBLN1	22q13.31
Fibulin-2 [51]		FBLN2	3p24–p25
Fibulin-3 [52]	EFEMP1	FBNL	2p16
Fibulin-4 [53,54]	MBP1, EFEMP2.	EFEMP2	11q13
Fibulin-5 [55,56]	DANCE, EVEC.	FBLN5	14q32.1
Fibulin-6 [57]	Hemicentin,	FBLN6	1q25.3

* BM90, basement membrane protein 90;EFEMP, EGF-containing fibulin-like extracellular matrix protein; MBP1, mutant p53-binding protein 1; DANCE, developmental arteries and neural crest epidermal growth factor (EGF)-like; EVEC, embryonic vascular EGF-like repeat-containing protein.

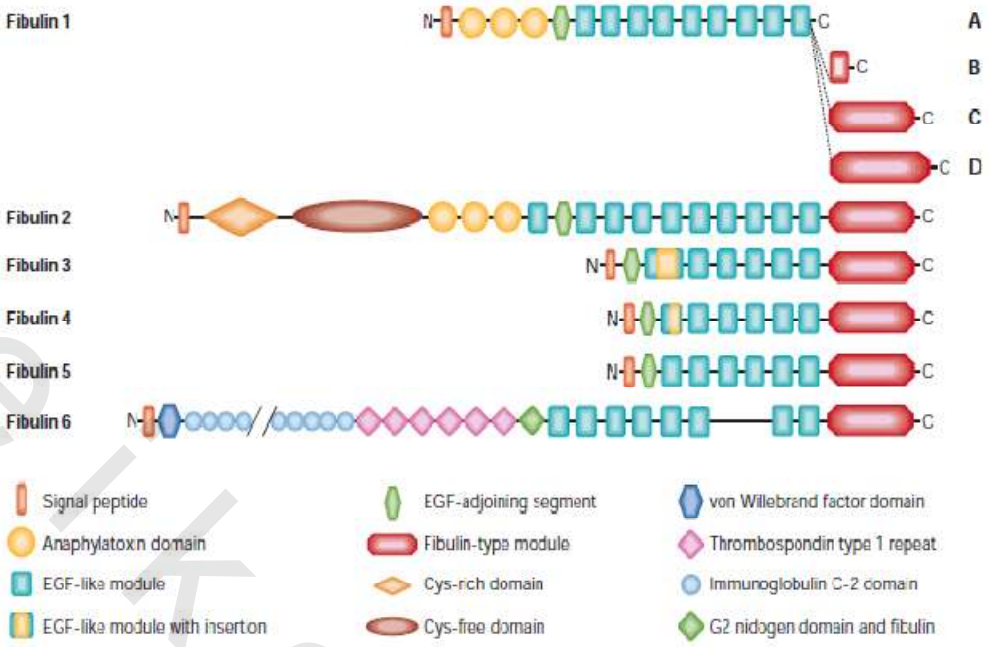

Fig. 1 | Modular structures of the fibulins. The most recent addition to the family, fibulin 6 (hemicentin), was originally identified in the nematode (Vogel & Hedgecock, 2001), with orthologues in other species (human, mouse and rat) having now been identified. Nine of the 48 immunoglobulin domains in fibulin 6 are shown (double slashes indicate where the omitted domains occur). Alternative splice variants are known for fibulins 1–4, albeit only variants for fibulin 1 (designated A–D) are displayed.

Figure (I): Modular structures of the fibulins.

The first member of the fibulin family — now referred to as fibulin-1 — was identified in affinity chromatography experiments.[47]The main component that was isolated from human placenta had a molecular mass of 90–100 kDa (from electrophoresis measurements),was shown to bind calcium and was considered to be a connecting element between β1 integrins and some cytoskeletal structures.

This view of fibulin-1 was soon changed because complementary DNA (cDNA) cloning showed the presence of a signal peptide and a modular structure that is characteristic of ECM proteins. This was consistent with other observations, which found that fibulin-1 in fibroblast cultures was deposited as extracellular fibrils and was also secreted into the blood in substantial amounts. [49]

The same protein could be also isolated from a tumor basement membrane, and was shown to be an ubiquitous basement-membrane protein by immunostaining. [50]Its incorporation into basement membranes is mediated by strong binding interactions with laminins and nidogens. [58]

A second, larger isoform — fibulin-2—was subsequently identified by cDNA sequencing [51, 59], and a disulphide-linked dimer of 200-kDa monomers was obtained by recombinant production in mammalian cells. [60, 61]

Three further closely related proteins —fibulin-3, fibulin-4 and fibulin-5 — of ~50–60 kDa were identified more fortuitously by a systematic search for new secreted proteins,

and were therefore initially named using various acronyms (TABLE 3) before they were classified as fibulins.[52,54]

Structure of fibulins

***Modular protein structure:**

Fibulin-1 was the first family member to be identified and it shows a distinct arrangement of typical ECM modules that are grouped together as domains I, II and III. [59,58] (FIG. I).

The amino terminal domain I consists of three anaphylatoxin-like (AT) modules, each ~40 residues long and containing four or six cysteines. The structure of an AT module was determined previously for the complement-derived anaphylatoxin C3a [62], and it was found to be a compact α-helical fold that is stabilized by three disulphide bridges in the pattern Cys1–4,Cys2–5 and Cys3–6(where Cys is cysteine).

The central domain II is composed of nine epidermal growth factor (EGF)-like modules, which are also ~40 residues long with three disulphide bridges (in the pattern Cys1–3, Cys2–4 and Cys5–6).Most of these EGF-like modules have a consensus sequence for calcium ligation, and they are known as calcium-binding EGF (cbEGF)-like modules. The carboxy-terminal domain III, which is ~120–140 residues long with only two extra cysteine residues, resembles a new protein module (FC; fibulin-type carboxyl terminus) that is shared by fibulins and fibrillins at the carboxyl terminus. [54]

A similar modular arrangement, with some modifications, is also characteristic of the carboxy-terminal segment of fibulin-2 (FIG.I), but, depending on the domain, it shows only 26–55%sequence identity with fibulin-1. [51]

Unique to fibulin-2 is a 400-residue amino-terminal domain — domain N— which can be subdivided into a cysteine-rich segment that is known as Na (12 cysteines in 150 residues) and a cysteine-free segment that is known as Nb.

The shortest isoforms identified so far in the family are fibulin-3, fibulin-4 and fibulin-5, and they share a central segment of five cbEGF-like modules and a carboxy-terminal domain III of variable sequence identity with the other fibulins [54](FIG. I). They have a unique amino-terminal domain I, which seems to be a modified cbEGF-like module that maintains its calcium-binding property (FIG. I).

**** Alternative splicing:***

The structure of most of the fibulins can be modified further by alternative messenger RNA splicing. This was initially shown for domain III of human fibulin-1, which can be completely eliminated in variant A or replaced by a short variant B (35 residues) in contrast to the longer variants C (117residues) and D (137 residues). [49, 52] (FIG. I). Variants A and B have so far only been detected at low levels in the human placenta.

Alternative splicing of fibulin-2 causes the elimination of a single cbEGF-like module in domain II (cbEGF3; FIG. I) and no variants have been detected for domain III.[51]

Five different splice variants have been described for human fibulin-3, which show a partial or complete deletion of the amino terminal domain I. [63]

A similar amino terminal truncation with the loss of the signal peptide also results in a weak level of expression of human fibulin-4. [64]

**** Calcium-binding properties:***

Many ECM proteins have been shown to bind calcium with moderate to high affinity and various types of protein modules can be involved in this ligation. A special class of EGF-like modules, which bind calcium and are referred to as cbEGF-like domains, are abundant in ECM proteins, including domain II of the fibulins (FIG. I).They are characterized by the consensus sequence D-X- D/N-E (where D is aspartate, X is any amino acid, N is asparagine and E is glutamate) before the first cysteine residue. [65]

Calcium binding has only been shown for fibulin-1 so far [47, 50], but it is probable that all fibulins bind calcium, as indicated by the loss of several binding activities in the presence of EDTA (ethylene diamine tetra-acetic acid). Furthermore, domain II of fibulin-1 is resistant to matrix metalloproteinases and other tissue proteases in the presence of calcium, and only a few bonds are cleaved in domain II of fibulin-2 in the same conditions.[66]The addition of EDTA, however, increases protease sensitivity in both cases.[66]

The structure of a cbEGF-like domain pair from fibrillin-1 has been determined and it showed that calcium is ligated by several residues both of the consensus sequence and of the loop between Cys2 and Cys4, as well as by a strong, hydrophobic intermodular contact.[65] (FIG. II).These intermodular interactions are thought to endow such tandem arrays with a high rigidity, which stabilizes the long, rod-like structure of fibrillins and fibulins.

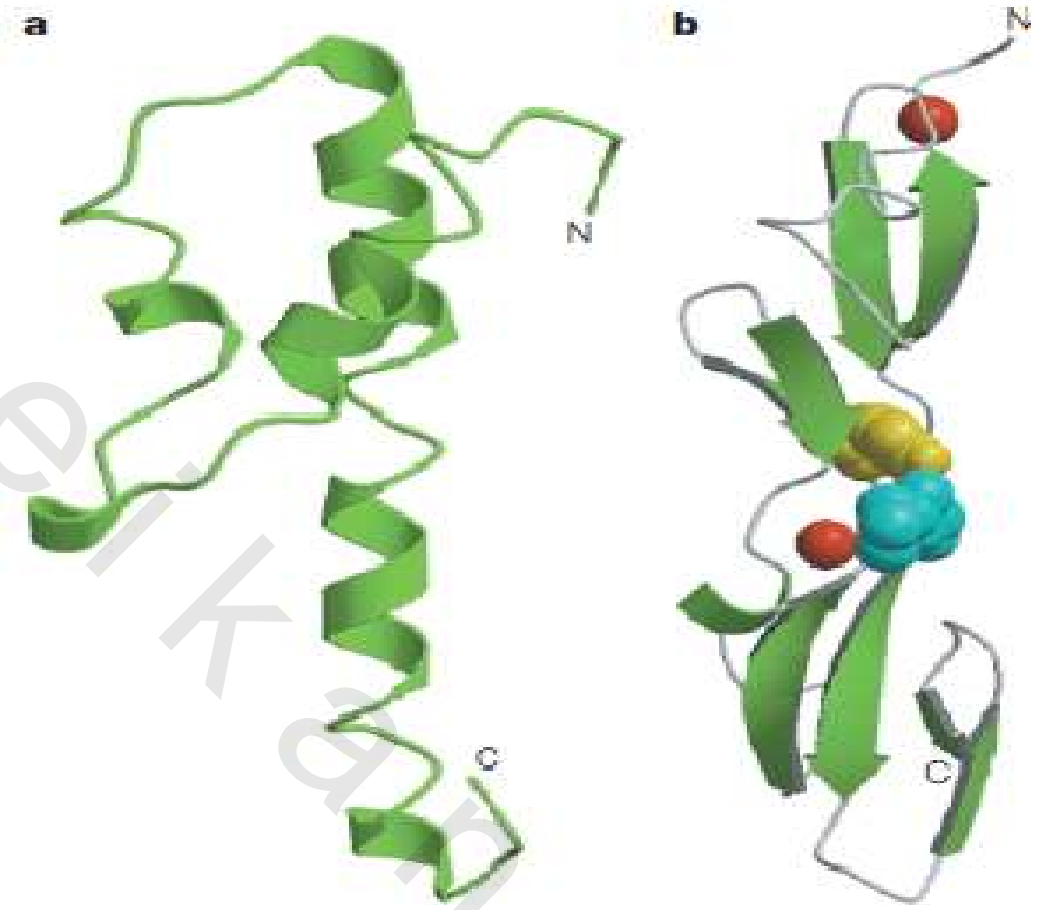

Figure (II): Potential structural models for anaphylatoxin-like modules and calcium-binding epidermal growth factor-like domain pairs in fibulins. A: The structure of complement-derived anaphylatoxin C3a as determined by X-ray crystallography. The analogous part of fibulin anaphylatoxin- like (AT) modules comprises only the upper amino-terminal half of anaphylatoxin C3a, which contains the three disulphide conds. This Figure was kindly provided by R. Huber (Max Planck Institute for Biochemistry, Martinsried, Germany). B: The NMR structure of a pair of calcium-binding epidermal growth factor (cbEGF)-like modules from fibrillin-a. The red balls indicate calcium bound to the amino-terminal consensus sequence in each module. A hydrophobic interaction at the interface between the modules is highlighted in yellow and cyan. This figure was kindly provided by A. K. Downing (Oxford (University, UK).

The fibulin genes:

The genes that encode the fibulins (*FBLN1*, *FBLN2*, *FBLN3*, *FBLN4* and *FBLN5*) have been mapped to different autosomal human chromosomes (TABLE 3). Their exon–intron structures have been determined and they explain the occurrence of alternative splice variants.

Expression and tissue deposition:

The most comprehensive analysis of expression and tissue deposition was reported for fibulin-1 in human adult tissues and cultured cell lines, and provided the first evidence for an association between fibulin-1 and elastic fibers. [67]

This association seems to occur through the amorphous elastin component of the elastic fibers and not through the microfibrillar structures (BOX 1). Fibulin-1 is also widely expressed in the ECM of various organs, in which it either associates with matrix fibers or with basement membranes. [50]

Fibulin-2 shows a more restricted tissue distribution, which only partially overlaps with that of fibulin-1. [51, 59]

Fibulin-3 and fibulin-4 are mainly detected in the walls of capillaries and larger blood vessels, in some basement membranes and, in part, in perineural tissue. [54]Fibulin-5 seems to be strongly expressed in large arteries and was localized to the elastic lamina close to endothelial cells. [68, 69]

Box 1: The structure and biology of elastic fibers

Elastic fibers are special forms of extracellular matrix assembly, which provide resilience to dynamic connective tissues and are crucial for the proper functioning of the lungs, arteries and skin. They can be readily identified at the ultrastructural level by their amorphous appearance, as they are frequently associated with numerous microfibrils. The amorphous core consists mainly of elastin, a highly hydrophobic protein that is generated from the soluble precursor tropoelastin (70 kDa) and is extensively cross-linked after the enzymatic oxidation of lysyl residues. Important components of the microfibrils are several fibrillin isoforms (~350 kDa, length ~150 nm), which have around 40 calcium-binding sites and some elastic properties. These microfibrils are believed to provide a scaffold for the deposition of elastin. More than 30 proteins, including some fibulins and collagens, have so far been identified as other components of some or all elastic fibers. [70] Elastic fibers are known to lose their regenerative potential during ageing and in certain obstructive diseases such as lung emphysema. Mutations in elastin and fibrillins are known to lead to various human disorders with skeletal, cutaneous, vascular and ocular malformations. [71, 72]

The synthesis of fibulin-1 starts at early stages of embryonic development and it is a constituent of most basement membranes in the avian embryo. [73] Although fibulin-2 synthesis starts somewhat later than fibulin-1 synthesis, both of these proteins are already at relatively high levels at the onset of organogenesis in the humans. [74] There are several interesting aspects of fibulin expression. Fibulin-1 can be detected in human serum [49, 50] at relatively high concentrations, whereas the concentration of fibulin-2 is 1000-fold lower,

which is more typical of an ECM protein. [51]Another interesting aspect is the deposition of fibulin-1 and fibulin-2 in various, but different, neuronal structures and the synthesis of fibulin-1 by several neuronal cell lines. [53,55, 67, 74-81]

Several immunohistological studies have shown a close co-localization of some fibulins and other prominent ECM proteins, which is indicative of molecular interactions. The results include the co-localization of fibulin-2 with fibrillin-1 in various tissues and of fibulin-1 and fibulin-2 with tropoelastin in the aorta. [82, 83] A similar co-localization was also observed between fibronectin and fibulin-1 and fibulin-2 in fibroblast cultures and was restricted, in the case of fibulin-2, to a particular class of amorphous microfibrils. [49, 84]

There might also be this latter co-localization in certain basement membranes such as in those of the testis, but it might not necessarily involve microfibrils. [85] Two types of microfibrils that contain fibulin-2 and either fibrillin-1 or fibronectin are anchored to the epidermal basement membrane and their abundance changes during skin regeneration. [86]

There could be similar switches in associations under pathological conditions. An upregulation of fibulin-1 has been detected during cutaneous wound healing [87], in sun-damaged elastotic skin and in contact dermatitis. [88,89] An increased synthesis of fibulin-1 has also been identified in ovarian carcinoma. [90]

Regulation of fibulins expression:

Information is gradually emerging concerning the mechanisms that regulate the expression of the fibulins during development or disease. Evidence indicates that steroids regulate the expression of fibulins 1, 2 and 3. Oestradiol stimulates fibulin-1C expression in ovarian tumor cells [91,92], and dexamethasone increases fibulin-1C expression in human eye trabecular meshwork cells. [93] Progesterone has been shown to stimulate the expression of fibulins 1 and 2 in human endometrial stromal cells. [94] Glucocorticoids downregulate the expression of fibulins-1 and 2 in bone marrow stroma [95] and estrogen represses the expression of fibulin-3 in Michigan Cancer Foundation-7 (MCF7) breast cancer cells. [96] Specificity protein (Sp) transcription factors are important in fibulin-1 expression. [97,98] Fibulin-1 transcription is activated by the ubiquitous Sp1 and Sp3, but not by the more tissue restricted Sp4. [97]

Fibulin-2 may be similarly regulated. [98] The fibulin-2 gene also contains two consensus cAMP-negative response elements. Interaction of cAMP activated liver X receptor-α with these enhancer elements results in increased fibulin-2 expression. [99]

Protein ligands and supramolecular structures:

The broad tissue distribution of the fibulins indicates that they have complex binding repertoires for other important ECM proteins.

Fibronectin microfibrils: The ECM and serum protein fibronectin was one of the first fibulin ligands to be identified by showing that it co-localizes with fibulin-1 [49] and fibulin-2[84] in microfibrils that are deposited in fibroblast cultures and by using various direct binding assays. [60,100,101]

Elastic fibers: Elastic fibers have a unique structure and special functions in tissues (BOX 1), and they have been shown to contain fibulin-1 [67] and fibulin-2. [82,83]

Basement-membrane proteins: Various basement membranes in many organs have been shown to contain fibulin-1 and/or fibulin-2 by immunohistology [58,74,102],and several important basement-membrane proteins — such as nidogen-1, some laminin isoforms, the heparan sulphate proteoglycan perlecan and collagen IV — have been identified as potential ligands for these associations.[58,60,103]

Lectican proteoglycans: Another group of highly potent fibulin ligands is represented by the lectican family of large chondroitin sulphate proteoglycans, which includes the cartilage-specific aggrecan, the ubiquitously deposited versican and the brain-specific neurocan and brevican. [104]

Fibulins in different organs and diseases:

- ***Fibulins in cardiovascular biology:***

Fibulins 1 and 2 are highly expressed during cardiac valvuloseptal formation. Both are produced by migratory cardiac mesenchymal cells that have trans-differentiated from endocardial cells. [76,78,79]

In developing and adult heart valves, fibulins 1 and 2 are prominently expressed and fibulin-4 is moderately expressed. [54,79]

Relatively little fibulin-3 is found in adult heart valves. [54] The fact that fibulin-1 deficiency does not result in overt valvuloseptal defects could indicate compensation by other fibulins. [105]

Fibulins are prominently expressed in blood vessels. During development, fibulin-1 is expressed by the primordial vascular smooth muscle cells (VSMCs) that associate with the ventral endothelium of the dorsal aorta. [106] It is not generally expressed in endothelial cells (ECs). [67]

Primordial VSMCs of the developing aortic-arch vessels also synthesize fibulin-2. In addition, fibulin-2 is expressed by coronary endothelial cells (ECs) that originate from epicardial cells, but it is not expressed by capillary ECs. [80] In adult blood vessels, pronounced fibulin-1 deposition occurs in the matrix that surrounds VSMCs and in the elastic lamina of arteries. [68]

Fibulin-3 expression is prominent in some capillaries, but not in large blood vessels.[54] It's also highly expressed in human umbilical vein ECs, but its expression is repressed during in vitro human capillary tube formation. [107]

Fibulin-4 is found in the medial layers of large veins and arteries and in some small capillaries. [54]

Fibulin-5 seems to be restricted to the arterial vasculature and is expressed predominantly by VSMCs of developing arteries and at low levels by VSMCs of adult blood vessels. [55] ECs also express fibulin-5, especially the pulmonary artery endothelium.[55,108]

- ***Fibulins and cancer:***

Human fibrosarcoma tumor cell lines show a trend towards a reduction or absence of fibulin-1D expression. [109]Fibrosarcoma cells that express fibulin-1D show reduced growth in vivo, as well as a lowered growth capacity in soft agar and a reduced ability to invade reconstituted basement membranes .[109]

Similarly, the ectopic expression of fibulin-1D inhibits the motility of breast carcinoma cells on fibronectin (FN). [110] The motility suppressive effects of fibulin-1D are attributed to a reduction in the cell adhesion and migration-promoting activity of FN. [110] Ectopic expression of fibulin-1D also inhibits transformation by the papilloma virus E6 gene. [111] The mechanism by which fibulin-1D regulates E6-mediated oncogenic activities might relate to the fact that these two proteins interact. [111] These findings support the conclusion that fibulin-1D acts as a tumor suppressor.

Elevated expression of fibulin-1 is associated with human breast tumors. [112,113] Also suggestive of fibulin-1 over-expression in breast carcinoma is the fact that breast cancer patients produce antibodies against fibulin-1. [112] These observations seem paradoxical in light of the evidence that fibulin-1D is a tumor suppressor. An explanation may come from findings that there is a trend towards increased expression of fibulin-1C compared with the D variant in ovarian carcinomas. [114] Levels of fibulin-1 splice variants have not been quantified in breast cancer but if fibulin-1C levels are elevated in breast tumors as in ovarian tumors, it would suggest that fibulin-1C opposes the action of fibulin-1D and promotes tumorigenesis. It is also possible that humoral immunity to fibulin-1 in breast cancer reflects the breakdown of fibulin-1D and concomitant loss of tumor suppression .In support of this; increased levels of fibulin-1 fragments have been reported in human breast tumors. [113]

Furthermore, findings from DNA microarray studies of lung adenocarcinomas show that fibulins-1 and 2 are consistently associated with matrix metalloproteinase 2 expression, a protein that promotes tumor invasion and metastasis. [115]

Fibulin-2 has been identified as one of 64 over-expressed metastasis-associated genes in solid tumors of diverse types. [116]

Fibulin-4 expression is elevated in human colon tumors [64], whereas cancers in other tissues tend to show down regulation of fibulin-5. [117]In contrast to the motility suppressive effects of fibulin-1D on fibrosarcoma cells, over-expression of fibulin-5 increases fibrosarcoma cell migration. [117]

- ***Fibulins in injury:***

The expression of several of the fibulins is induced in response to injury. Fibulin-1 expression is increased in a murine model of cardiomyopathy that is caused by increased Gi-receptor signaling. [118]

In sun-damaged skin elastosis, fibulin-2 deposition in association with elastic fibers is greatly increased. [88] Fibulin-2 expression is also increased in the early phase of streptozotocin-induced diabetic glomerulosclerosis. [119]

The expression of fibulin-5, which is low in adult arteries, is activated in medial and neointimal VSMCs in response to vascular injury [55], as well in lung vasculature in response to hyperoxia [108] and in atherosclerotic plaques. [55] Transforming growth factor-β (TGF-β), which has a key role in vascular injury response, stimulates fibulin-5 expression.[117] A similar profile of protein kinase activation has also been observed in response to fibulin-1 stimuli. [110]

Over expression of fibulins 5 and 3 increase fibroblast DNA synthesis. [117,120] Over-expression of fibulin-4 in macrophages also promotes DNA synthesis [121], and fibulin-4 expression is augmented in macrophages by lipo-polysaccharide treatment, which suggests a role in response to sepsis. [121]

A recent study has shown that patients with unstable angina pectoris and acute myocardial infarction have significantly reduced levels of plasma fibulin-1. [122] This has led to speculation that plasma fibulin-1 may be transferred to or consumed in or around the atherosclerotic lesion. Indeed, fibulin-1 is incorporated into fibrin clots that are associated with atherosclerotic lesions. [123] The significance of fibulin-1 in the development of atherosclerosis is not yet known, but plasma fibulin-1 could be important as a risk factor for cardiovascular diseases and atherosclerosis progression.

- **Effects on cellular activities:**

Interactions with integrins. Because of their broad occurrence in the ECM, several of the fibulins were also studied for their ability to promote integrin-mediated cell adhesion and migration. Mouse fibulin-2 was shown to bind to purified platelet αIIbβ3 and αvβ3, which are both RGD-dependent integrins (a specific group of cellular integrin receptors that bind to the arginine-glycine-aspartate (RGD) sequences of their ligands), and this interaction was confirmed in adhesion assays with activated platelets and other cells.[124] The RGD sequence in the N domain of mouse fibulin-2 was shown to be involved in this interaction, but this RGD sequence is not conserved in human fibulin-2, which still binds the αIIbβ3 integrin. Together, the data indicate that there is a role for fibulin-2 in haemostatic control. Fibulin-1 lacks RGD sequences and had no activity in cell-adhesion assays. [110,124]However, fibulin-1 inhibited cell adhesion to, and migration on, fibronectin. The binding of fibulin-1 does not, however, block the integrin-binding sites of fibronectin, which led to the proposal that the formation of this complex generates a new anti-adhesive site, which repulses cellular interactions rather than promotes them. [110] Finally, a single RGD site in the amino-terminal domain I of fibulin-5 has also been shown to be cell adhesive, and it interacts, in particular, with αvβ3, αvβ5 and α9β1 integrins. [56.68]

Effect on cell proliferation. There is an increasing amount of data that indicate that most fibulins have the ability to interfere with several cellular activities, and these data were interpeted to reflect their control of cellular proliferation and malignant transformation. Most of these data are, however, preliminary and, in part, controversial, and no general concept has emerged for their interpretation. This is perhaps best illustrated by a recent study on fibulin-5, which came to the conclusion that regulation occurs in a context specific manner and depends on the cell type and assay conditions used. [108] The first evidence for a cellular proliferation activity was described for fibulin-3, which is up-regulated in senescent and Werner syndrome fibroblasts (A premature ageing disorder that is inherited in an autosomal recessive mode. The clinical symptoms can include short stature, wrinkled skin, baldness, cataracts and muscular atrophy), as well as in quiescent young fibroblasts.[63] This indicated that there was an effect on DNA synthesis, but the data have so far remained controversial. A similar change in quiescent fibroblasts has also been reported for fibulin-4 [54], although fibulin-4 has also been shown to stimulate growth. [122] A few further interesting activities have been reported for fibulin-1 including modulation of neurite outgrowth through binding to β-amyloid precursor protein (APP) [82], the binding of growth factors such as connective-tissue growth factor (CTGF) [125] and the regulation of signal transduction by inhibiting the phosphorylation of the extracellular-signal-regulated kinase (ERK). [110] The latter activity is also shared by fibulin-5. [117] The cellular receptors that are involved remain to be identified.

Fibulin-1 (FBLN-1):

Structure:

Fibulin-1 was the first family member to be identified and it shows a distinct arrangement of typical ECM modules that are grouped together as domains I, II and III. [49, 58](FIG. I).

The amino terminal domain I consists of three anaphylatoxin-like (AT) modules, each ~40 residues long and containing four or six cysteines. The structure of an AT module was determined previously for the complement-derived anaphylatoxin C3a [62] , and it was found to be a compact α-helical fold that is stabilized by three disulphide bridges in the pattern Cys1–4,Cys2–5 and Cys3–6 (where Cys is cysteine).

The central domain II is composed of nine epidermal growth factor (EGF)-like modules, which are also ~40 residues long with three disulphide bridges (in the pattern Cys1–3, Cys2–4 and Cys5–6). Most of these EGF-like modules have a consensus sequence for calcium ligation, and they are known as calcium-binding EGF (cbEGF)-like modules.

The carboxy- terminal domain III, which is ~120–140 residues long with only two extra cysteine residues, resembles a new protein module (FC; fibulin-type carboxyl terminus) that is shared by fibulins and fibrillins at the carboxyl terminus. [54]

The synthesis of fibulin-1 starts at early stages of embryonic development and it is a constituent of most basement membranes in the avian embryo. [73]

Fibulin-1 homomultimerizes and interacts with various ECM components such as fibronectin (FN) [100] , laminin subunits alpha-1 and alpha-2 (LAMA1 and LAMA2), nidogen (NID), Aggrecan core protein (ACAN), versican core protein (CSPG2) and type IV collagen proteins. [48] Fibulin-1 also interacts with amyloid beta A4 (APP) [82], insulin-like growth factor-binding protein 9 (NOV) [125], fibrinogen (FGB) [56], and human papillomavirus (HPV) type 16, 18, 31 proteins. [111]

Function:

Fibulin-1 is incorporated into FN-containing matrix fibers. It plays a role in cell adhesion and migration along protein fibers within the ECM and is important for certain developmental processes. Fibulin-1 contributes to the supramolecular organization of ECM architecture, in particular to that of the basement membrane. It is implicated in cellular transformation and tumor invasion, and can behave both as an oncosuppressor and oncogene depending on tissue environment. It also plays a role in hemostasis and thrombosis owing to its ability to bind fibrinogen and incorporate into clots and plays a significant role in modulating the neurotrophic activities of APP, particularly soluble APP.[48]

Isoforms of fibulin-1:

Four isoforms of FBLN-1 have been identified to date in humans, designated FBLN-1A, 1B, 1C, and 1D (Figure III). [68] FBLN-1C negatively regulated proliferation in human osteoblasts, so the actions of the FBLN-1 isoforms are likely to be cell type specific. [110] FBLN-1 has been reported to decrease [126] and increase [127] wound repair in other organs.

The differential regulation of the specific isoforms of FBLN-1 indicates that they may have opposing or compensatory effects. [114] FBLN-1D mRNA expression reduced in lysates from asthma derived bronchial biopsies compared with those derived from non-asthmatics. [128] FBLN-1C appears to have different properties to the other FBLN-1 isoforms. [114]

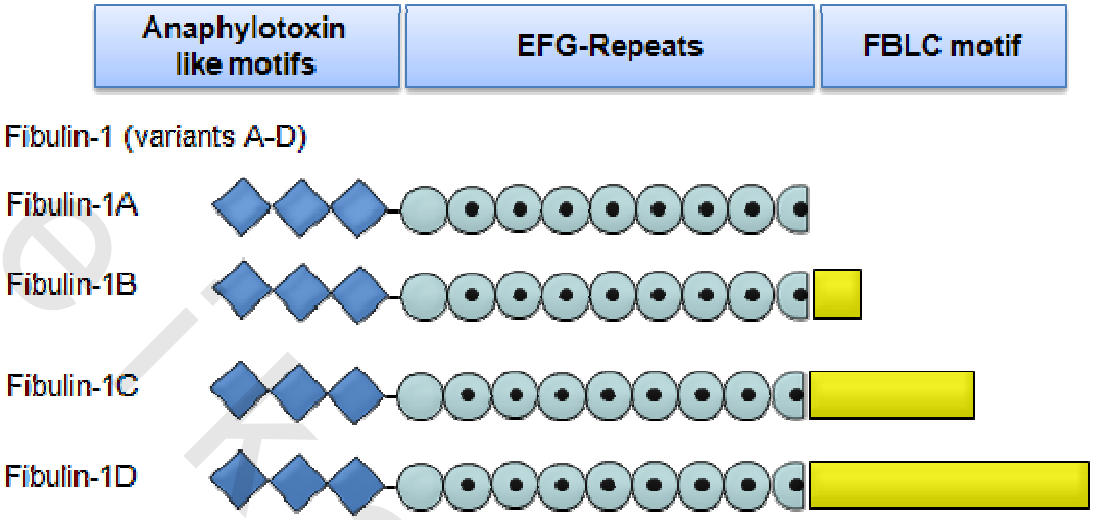

Figure (III): ***Isoforms of fibulin-1.***

Genetic location:

22q13.31 (Figure IV).

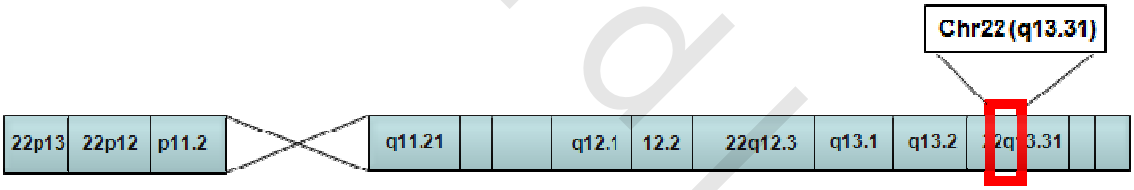

Figure (IV): ***Genetic location of fibulin-1.***

Fibulin-1 is an extracellular matrix (ECM) and blood glycoprotein. It is a member of the fibulin glycoprotein family which includes 7 proteins thought to function as bridges in the organization of ECM supramolecular structures. [48, 49]

Description:

Sequence length: 97, 71 Kb, 20 exons, max. exon length 818, min. exon length: 50. Number of SNPs: 1038. Four splice variants have been identified which differ in the 3' end and encode different isoforms (A, B, C and D). [129]

Variant D: This variant is considered the canonic transcript form: 2947 bp;

- Including exons 18, 19 and 20;
- Lacking exons 15 and 16.

Variant B: 2530 bp;
- Including exon 17;
- Lacking exons 16,18, 19 and 20.

Variant A: 2350bp;

- Including exon 15;
- Lackings exons 16 to 20.

Variant C: 2313bp;

- Including exon 16;
- Lacking exons 15 and 17 to 20.

Mutation:

- Location: exon 19 (acceptor splice site) (Figure V)
- Substitution: G-A
- Phenotype: Bernard-Soulier syndrome

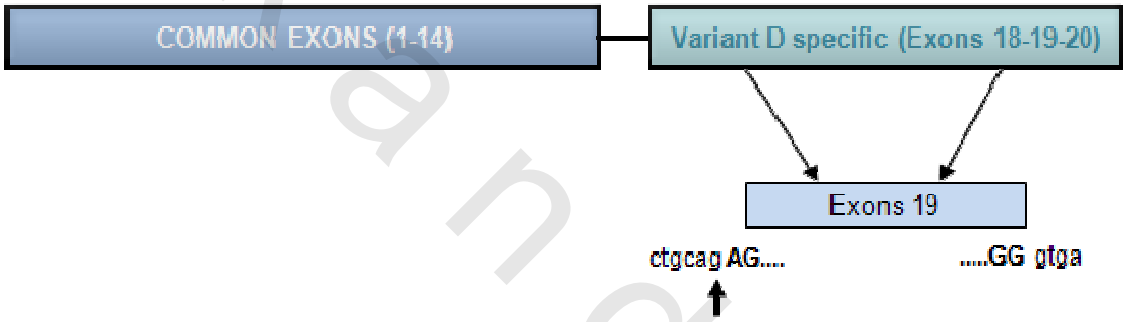

Figure (V): ***Mutation in fibulin-1 gene.***

Expression and tissue deposition:

The most comprehensive analysis of expression and tissue deposition was reported for fibulin-1 in human adult tissues and cultured cell lines, and provided the first evidence for an association between fibulin-1 and elastic fibers.[67] This association seems to occur through the amorphous elastin component of the elastic fibers and not through the microfibrillar structures (BOX 1).

Fibulin-1 is also widely expressed in the ECM of various organs, in which it either associates with matrix fibers or with basement membranes. [50] Fibulin-1 can be detected in human serum [49,50] at relatively high concentrations (10–50 µg/ml).

Regulation of fibulin-1 expression:

Steroids regulate the expression of fibulin-1. Estradiol stimulates fibulin-1C expression in ovarian tumor cells [91,92] , and dexamethasone increases fibulin-1C expression in human eye trabecular meshwork cells. [93]

Progesterone has been shown to stimulate the expression of fibulin-1 in human endometrial stromal cells. [94]

Glucocorticoids downregulate the expression of fibulin-1 in bone marrow stroma.[95]

Specificity protein transcription factors (Sp transcription factors) are important in fibulin-1 expression. [97.98] Fibulin-1 transcription is activated by the ubiquitous Sp1 and Sp3, but not by the more tissue restricted Sp4. [97]

Specific fibulin-1 ligands:

A few more interactions have been described for fibulin-1, which do not seem to be shared by fibulin-2. A weak affinity (Kd = 3 μM where Kd is the equilibrium dissociation constant of bimolecular reactions.) was described for the fibrinogen βB chain, and this interaction causes fibulin-1 to associate with, and to inhibit the formation of, fibrin clots.[123,130] However, fibulin-1-deficient mice that lack the circulating form of this protein show no obvious defect in coagulation [105],which indicates that this function is not crucial. Two more ligands were initially identified by yeast two-hybrid screening and were subsequently confirmed using recombinant fibulin-1 in other binding assays. The first ligand was connective-tissue growth factor (CTGF), as well as related members of this protein family, which correlates well with the co-expression of fibulin-1 and these growth factors. [125] The other ligand was β-amyloid precursor protein (APP), which binds to cbEGF3–7 of fibulin-1 in a calcium-dependent fashion. [81] This interaction inhibits APP stimulation of neurite outgrowth and neuronal stem cell proliferation. A similar role *in vivo* is indicated by the presence of fibulin-1 in some neurons [67] , but it is not yet known whether fibulin-1 is also incorporated into amyloid deposits in the brain.

Fibulin-1 suppression of fibronectin-regulated cell adhesion and motility:

Cell movement is a fundamental feature of many normal and pathological processes, including developmental morphogenesis, inflammation, wound healing and metastasis. The movement of cells in these contexts is largely dependent on cell interactions with specific components of the extracellular matrix (ECM). [131,132] Cell-ECM interactions promote, sustain, orient and inhibit cell movement. [133] The literature is replete with examples of ECM proteins that promote cell motility, such as fibronectin (FN) [134], laminin [135], thrombospondin-1 [136], and collagens. [137,138] A growing number of ECM proteins that negatively regulate cell motility have also been described, including tenascin, osteonectin, aggrecan and versican. [139-142]

Fibulin-1 is found in association with ECM structures such as microfibrils, basement membranes, elastic fibers and fibrin. [67,123] The association of fibulin-1 with these ECM structures is probably based on its ability to bind ECM proteins such as FN, laminin, nidogen, endostatin (C terminal domain NC1 of collagen XVIII), tropoelastin and fibrinogen. [58, 83,100,123,143] Several studies suggest that the interaction between fibulin-1 and FN might be of particular importance. For example, fibulin-1 can be detected with FN in focal adhesion sites an hour after seeding fibroblastic cells on FN-coated surfaces. [47, 49] Within 12-24 hours after seeding of such cells, fibulin-1 can be found decorating FN containing microfibrils. [49] Treatment of fibroblastic cells with antagonists of FN matrix assembly, such as integrin antibodies, blocks the incorporation of fibulin-1 into ECM fibrils. [144,145] Furthermore, cells that fail to assemble a FN matrix do not incorporate

fibulin-1 into ECM fibrils. [144] The association of fibulin-1 and FN has been demonstrated in vivo in embryonic tissues such as the cardiac cushions [77] and in tissues of the adult such as bone marrow stroma. [146]Given the close relationship between FN and fibulin-1, it was explored the possibility that fibulin-1 functions to regulate important biological activities of FN including promotion of cell adhesion and motility.

Fibulin-1 is an inhibitor of in vitro cell adhesion and motility. The in vivo significance of this activity remains to be determined. However, a motility-suppressing role for fibulin-1 is consistent with observations showing that, during development, fibulin-1 expression is found in association with certain migratory populations of cells. [63] For example, endocardial cushion mesenchymal cells have fibulin-1 on their surfaces and deposited in adjacent ECM.[63, 64,77] Similarly, fibulin-1 is expressed by epicardial cells that differentiate into cells that migrate into the myocardium and endocardial cushions. [64,102] In addition, fibulin-1 expression has been observed in and around cells migrating from the neural crest. [63] An in vivo motility-suppressing role for fibulin-1 would be expected to extend beyond the phase of embryonic development, because fibulin-1 is also a component of the ECM of many adult tissues. [67,146] For example, fibulin-1 is prominently associated with the ECM that surrounds vascular smooth muscle cells [67] , perhaps acting to suppress movement of quiescent smooth muscle cells or leukocytes. Likewise, fibulin-1 has been shown to be deposited into fibrin-containing thrombi [123] and might modulate the motility of cells that infiltrate clots and thus participate in remodelling of provisional matrices of wounds. Additionally, fibulin-1 has been found in peritumour stroma of human ovarian cancer [91] and shown to suppress the motility of ovarian carcinoma cells in vitro. [92]

Furthermore, fibulin-1 has been shown to also suppress the growth of fibrosarcoma tumours in nude mice, presumably through its ability to suppress fibrosarcoma cell invasiveness. [109]

Fibulin-1, which is normally present in basement membranes and loose connective tissues, might suppress tumor cell invasion. Fibulin-1 specifically suppresses the motility promoting activity of FN. The underlying mechanism for this activity remains uncertain. In this regard, it is important to point out that fibulin-1 binds to FN within type III repeat module13. [100] This repeat module contains the major cell surface heparan sulfate proteoglycan (HSPG)- binding domain of FN [147,148,149], and an a4b1 integrin binding site.[150,151] The type III13 module is also near to the other integrin-binding sites in FN, the arginine-glycine-aspartate sequences(RGD) site contained within the type III10 module [152] and the alternatively spliced V (IIICS) region. [153]

Given the location of the fibulin-1-binding site in the vicinity of binding sites for integrins and cell surface HSPGs, the possibility that fibulin-1 might modulate the interaction of integrins and proteoglycans with FN, which are important for cellular adhesion and motility was evaluated.

It is plausible that fibulin-1 binding to FN acts to expose the cryptic anti-adhesive site in FN that has recently been located in the Hep-2 region of FN, the same region that contains the fibulin-1-binding site. [154] This cryptic anti-adhesive site is exposed following conformational changes in FN induced by heparin binding or urea denaturation. [154]

Mmajor histocompatibility complex (MHC) phosphorylation has been postulated to initiate spreading by releasing F-actin from actinomyosin complexes, allowing it to

reassemble within lamellipodial protrusions. [155]This results in a loss of actinomyosin-based contractility, leading to cell spreading. Fibulin-1 suppresses cell spreading and MHC phosphorylation. Cells attached to surfaces of FN plus fibulin-1 have a rounded morphology, generally lack lamellipodia and display a lower rate of spreading than cells on FN substrata. Fibulin-1 was also found to inhibit FN mediated activation of ERK. It is not known whether there is a relationship between ERK and MHC phosphorylation. MHC phosphorylation has been shown to involve an influx of extracellular calcium and activation of a calmodulin dependent kinase (CaM kinase). [155] Franklin et al [156] have shown that increases in intracellular calcium induce activation of ERK1/2 in human T cells via activation of CaM kinase. Inhibition of CaM kinase has been shown to prevent ERK1/2 activation. [157] Fibulin-1 might reduce intracellular calcium levels, thus reducing the activation of both ERK and MHC. It might accomplish this by modulating a5b1-FN signalling, which has been shown to regulate a tyrosine phosphorylation cascade that controls the function of the L-type calcium channel. [158]

Fibulin-1 in diseases:

Several studies have reported that fibulin-1 is over-expressed in various human neoplasias and it is implicated in processes such as invasion, motility, and in vivo tumor growth. [109,110,111,113,114] Fibulin-1 inhibits in vitro adhesion and motility of various carcinoma cell lines. [110]

Breast cancer:

Fibulin-1 was found aberrantly expressed in ~35% of 528 human primary breast cancers. Its expression is associated with improved survival in patients with lymphoid infiltrate at the tumor site [159], suggesting a possible involvement in triggering a protective antitumor immune response. Fibulin-1 induces specific B- and T-cell-mediated responses in breast cancer patients. [112,159] Its over-expression can serve as a tool for early detection of breast cancer [160] and acts to promote breast cancer cell survival during doxorubicin treatment. [161] In a series of 36 primary breast carcinomas, the expression of mature fibulin-1 polypeptide (100 kDa) did not correlate with estrogen receptor-alpha (ERα) or progesterone receptor (PR) levels, whereas a positive correlation was found between fibulin-1 processing (50 kDa fragment) and ERα and PR protein levels. [113]

Ovarian cancer:

The molecular mechanisms involved in ovarian carcinogenesis remain unclear, but growing evidence indicates that estrogens promote progression of ovarian cancer and increase expression levels of some secreted proteins. Differential over-expression of ERα versus -ERβ has been demonstrated during ovarian carcinogenesis [91,114], suggesting that estrogen-induced proteins, including fibulin-1, may act as ovarian tumor-promoting agents. In ovarian tissues and cancer cell lines, fibulin-1C and -1D are the predominant forms, whereas fibulin-1A and -1B are weakly expressed. An increased fibulin-1C:-1D mRNA ratio in ovarian cancer cells as compared to that in normal ovaries has been observed, suggesting that the C variant is the main one involved in ovarian carcinogenesis. Fibulin-1C over-expression might provide a clue in understanding the putative role of estrogens in ERα-promoted ovarian tumor progression. [114] In addition, quantitative proteomic analysis integrated with microarray data reveals that fibulin-1 is one of the ECM-related molecules important for chemotherapy response. [162]

Hypermethylation of fibulin-1 gene in epithelial cancers:

It has been previously reported both in gastric and prostate cancer models that fibulin-1 acts as a tumor suppressor gene and is regulated by promoter hypermethylation.[163,164] Very recently, also in hepatocellular carcinoma (HCC) model, a down-modulation of fibulin-1 expression caused by its promoter hypermethylation has been proposed as a marker of HCC progression. [165]

Mesenchymal cancers

Osteosarcoma:

Recently, a distinct gene expression pattern of osteoblastic and non osteoblastic osteosarcoma groups has been proposed. In particular, they found significantly modulated the expression of several genes involved in the formation of extracellular matrix, including fibulin-1, already known to be involved in cell morphology modulation, growth and invasion of sarcoma cells [109] and suggest that fibulin-1 could be exploited as potential therapeutic target in the future. [166]

Hematological cancers

Lymphoma:

Using a chemical proteomic approach in Hodgkin Lymphoma (HL), Kischel and coauthors identified several extracellular matrix proteins, including fibulin-1, over-expressed in HL. [167] Based on its high expression levels in HL biopsy samples, fibulin-1 has been suggested as a potential targets for HL immunotherapy.

Synpolydactyly:

Synpolydactyly is a rare genetic disorder characterized by malformations in the hands and feet, with abnormalities including increased finger and toe numbers and fusion of digits into a single digit. Molecular analysis of the reciprocal chromosomal translocation t (12; 22) (p11.2; q13.3) co-segregating with a complex type of synpolydactyly indicated involvement of an alternatively spliced exon of the fibulin-1 gene (FBLN1 located in 22q13.3). Investigation of the possible functional involvement of the fibulin-1 protein in the observed phenotype showed that fibulin-1 is expressed in the ECM in association with the digits in the developing limb. [168] Thus, t (12; 22) might result in haplo-insufficiency of the fibulin-1D variant, leading to the observed limb malformations.

Bernard-Soulier syndrome:

Bernard-Soulier syndrome is an autosomic-dominant disease that causes alterations of the megakaryocyte/platelet lineage and is characterized by bleeding tendency, giant blood platelets and low platelet counts. An unexpected mutation in the splice acceptor site of fibulin-1 exon 19 was found in affected individuals of the Israeli Fechtner family. In all affected individuals from eight families, expression of fibulin-1 variant D was inhibited by putative antisense RNA [169], raising the possibility that these autosomal-dominant giant platelet syndromes are associated with aberrant antisense gene regulation of fibulin-1.

Placenta dysplasia:

Placental dysplasia is a rare human placental disorder in which the placenta is enlarged and contains cystic villi and dilated vasculature. A significant correlation was observed between fibulin-1 gene over-expression and murine placental overgrowth [(170)], suggesting that the gene and its product have a functional role in placenta development.

Morphogenesis of neural crest-derived structures:

A significant negative correlation between fibulin-1 gene expression and some morphogenic anomalies of neural crest-derived structures in mice has been reported. [(171)] Such fibulin-1-deficient mice exhibit cardiac ventricular wall thinning and ventricular septal defects, with double outlet right ventricle or overriding aorta, as well as aortic arch arteries anomalies, hypoplasia of the thymus and thyroid, underdeveloped skull bones, malformations of cranial nerves and hemorrhagic blood vessels in the head and neck. The spectrum of malformations is consistent with a role for fibulin-1 in neural crest cell-dependent development of these tissues.

Acute aortic dissection:

Acute aortic dissection (AAD) is a tear in the wall of the aorta that causes blood to flow between the layers of the wall of the aorta and force the layers apart. AAD is a life-threatening condition with high mortality and a mostly unclear pathophysiological mechanism. Down-regulation of fibulin-1 noted in AAD compared to control samples might determine a weakening of ECM in the aorta and/or interfere with the transmission of cellular signals causing AAD. [(172)]

Atherosclerotic lesions:

Fibulin-1 deposits were found in association with fibrinogen in atherosclerotic lesions and in regions containing fresh thrombi. By contrast, fibulin-1 was not detected in sclerotic regions and low levels were associated with smooth muscle cells within and outside lesions. [(173)] Further, an accumulation of fibulin-1 deposits were found in the arterial wall and in plasma of patients with type 2 diabetes and appears to be a factor associated with arterial extracellular matrix alterations. [(174)]

Thrombosis:

Thrombosis, the formation of a blood clot (thrombus) inside a blood vessel, obstructs blood flow through the circulatory system. Analyses of blood plasma revealed an interaction between fibulin-1 and fibrinogen [(123)], pointing to potential new roles for fibulin-1 in hemostasis as well as thrombosis.

Infection disease:

Streptococcus Pyogenes, which belongs to the group A of streptococcus, has been found to interact with the host fibulin-1 through its major serum opacity factor (SOF) receptor that is a major fibronectin binding protein involved in adhesion to host cells. The SOF-fibulin-1 interaction can lead to initial bacterial adhesion. [(175)]

Fibulin-1 in bronchial asthma:

Asthma is a chronic inflammatory disorder of the airways characterized by variable and reversible airflow obstruction and airway hyper-responsiveness (AHR). A key feature of asthmatic airways is remodeling which involves thickening of the airway wall, altered deposition of extracellular matrix (ECM) proteins [176,177] and increased airway smooth muscle (ASM) mass. These structural changes may result from an aberrant repair process in the lung, which includes increased proliferation of the ASM cells. [178,179] Whilst current treatments control the symptoms of asthma, they are unable to fully prevent or reverse airway remodeling.

The ECM maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function. [180] The ECM deposited by asthma derived ASM cells is altered such that increased amounts of collagen I and laminin [181-183], as well as fibronectin (FN) are produced which mediate a range of cellular interactions including migration, growth and differentiation.

Levels of the profibrotic cytokine transforming growth factor β (TGF-β) are elevated in the bronchoalveolar lavage (BAL) fluid in asthma [184], and are increased in bronchial tissue. [185] TGF-β stimulated FN deposition is also enhanced in asthma derived bronchial epithelium and fibroblasts. [186,187]

Fibulin-1 (FBLN-1), a secreted glycoprotein, assists in stabilizing the ECM. It associates with FN and a variety of other ECM proteins including laminin and fibrinogen.[188] Mice deficient in FN and FBLN-1 die perinatally due to abnormal lung development. [105] FBLN-1 expression has been reported in human lung tissue using microarray technology, however, FBLN-1 was not verified by PCR or at the protein level, nor were functional studies carried out. [128,189,190] In addition, Laprise et al., reported reduced FBLN-1D expression in asthma derived bronchial biopsies compared with those derived from non-asthmatics. [128]