What people with Down Syndrome can teach us about cardiopulmonary disease

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This review summarises the cardiopulmonary and immune challenges faced by individuals with Down syndrome http://ow.ly/tlGU306iMkG

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ABSTRACT Down syndrome is the most common chromosomal abnormality among live-born infants. Through full or partial trisomy of chromosome 21, Down syndrome is associated with cognitive impairment, congenital malformations (particularly cardiovascular) and dysmorphic features. Immune disturbances in Down syndrome account for an enormous disease burden ranging from quality-of-life issues (autoimmune alopecia) to more serious health issues (autoimmune thyroiditis) and life-threatening issues (leukaemia, respiratory tract infections and pulmonary hypertension). Cardiovascular and pulmonary diseases account for ∼75% of the mortality seen in persons with Down syndrome. This review summarises the cardiovascular, respiratory and immune challenges faced by individuals with Down syndrome, and the genetic underpinnings of their pathobiology. We strongly advocate increased comparative studies of cardiopulmonary disease in persons with and without Down syndrome, as we believe these will lead to new strategies to prevent and treat diseases affecting millions of people worldwide.

Introduction

Down syndrome is the most common chromosomal abnormality worldwide, with an incidence of between 1 in 700 and 1 in 800 live births [1]. Across Europe, ∼9000 babies are born with Down syndrome annually. It is a multisystem condition caused by the presence of a third copy of part or all of human chromosome 21 (Hsa21). Invariably, Down syndrome is associated with a spectrum of craniofacial abnormalities, hypotonia and cognitive impairment, as well as early-onset Alzheimer's dementia [2]. Other medical problems are common in persons with Down syndrome: gastrointestinal malformations, congenital heart defects, respiratory disease, autoimmunity, thyroid dysfunction and haematological disorders [3] (figure 1). Persons with Down syndrome are resistant to the development of solid tumours and coronary atherosclerotic disease (CAD). Unfortunately, the mechanisms responsible for predisposition or resistance to these conditions are poorly understood. ∼45–50% of all newborns with Down syndrome have a congenital heart defect (CHD), usually atrioventricular septal defect [4], depending on ethnicity and sex [5]. Despite elevated risk factors of lipid metabolism [6] and obesity [7], the incidence of

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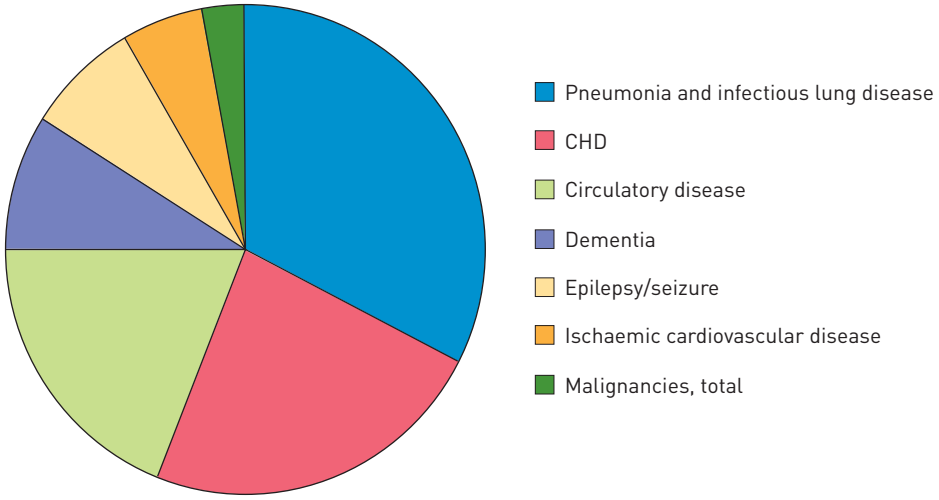

FIGURE 1 Heart and lung diseases are the leading causes of death for persons with Down syndrome. Pneumonia and infectious lung disease, congenital heart defect (CHD) and circulatory disease (vascular diseases not including CHD or ischaemic heart disease) account for ~75% of all deaths in persons with Down syndrome. Interestingly, ischaemic cardiovascular disease accounts for only ~7% of deaths in Down syndrome, compared to the typical population mortality rate of ~30% (not shown). Reproduced and modified from [38] with permission from the publisher.

TABLE 1 Respiratory complications of Down syndrome

Common	
Pneumonia/recurrent respiratory infection	Immune evaluation Consider bronchoscopy to evaluate for anatomical abnormalities (*e.g.* tracheal bronchus) Evaluate swallowing function for dysphagia
Sleep-disordered breathing	Polysomnogram if any evidence of snoring, adenoidal or tonsillar hypertrophy, poor sleep pattern, obesity or pulmonary hypertension
Laryngomalacia	Consider flexible bronchoscopy to evaluate severity, even in clinically mild cases If moderate to severe, consider polysomnogram to evaluate for obstructive sleep apnoea Evaluate swallowing function
Tracheobronchomalacia	Consider in patient with noisy breathing, chronic cough, persistent or atypical wheezing Refer for flexible bronchoscopy
Tracheal bronchus	Consider in patients with recurrent or persistent right upper lobe pneumonia
Pulmonary hypertension	Consider in all patients with upper airway obstruction or unexplained hypoxia Echocardiogram and cardiology consultation
Subpleural cysts	Usually incidental finding on chest contrast tomography Can usually be managed with close observation
Subglottic stenosis	Refer for bronchoscopy
Less common	
Post-obstructive pulmonary oedema	Anticipate in patients who require upper airway surgery
High-altitude pulmonary oedema	Persistent pulmonary hypertension of the newborn
Complete tracheal rings	Diagnosed by bronchoscopy
Pulmonary haemorrhage	Consider in patients with recurrent abnormal chest radiographs, unexplained hypoxia or anaemia Refer for bronchoscopy
Interstitial lung disease	Consider in patients with unexplained hypoxia

atherosclerotic disease in adults with Down syndrome is low [8, 9]. Thyroid-related cardiac dysfunction is common in Down syndrome [10]. Lung disease accounts for 54% of hospital admissions in Down syndrome [11], and average length of admission is two to three times longer than in individuals without the syndrome [12]. For persons with Down syndrome aged <3 years, respiratory illnesses are the most common cause of hospital admissions [12]. Persons with Down syndrome have increased frequency of respiratory tract infection [13] and acute respiratory distress syndrome [14]. Respiratory disease is the most common cause of death in persons with Down syndrome of any age [15]. There is a large population of persons with Down syndrome in Europe, the United States, and worldwide. A large Down syndrome population, combined with the high incidence of cardiopulmonary disease in Down syndrome, is therefore associated with significant morbidity, mortality and cost. We have a unique opportunity to advance the science and treatment of cardiopulmonary disease for all individuals by studying these diseases in the context of Down syndrome.

In this review we highlight the unique opportunity provided by persons with Down syndrome to the broader biomedical research community. CAD is the leading global cause of death, and caused >1.8 million deaths (42% of total deaths) in 2014 in Europe alone [16], but for reasons that are not clear, atherosclerotic disease and CAD are rare in Down syndrome. Respiratory diseases, excluding lung cancer, are responsible for ∼15% of all deaths in Europe [17]. Of the 10 leading causes of infant mortality, four were lung diseases, accounting for ∼30% of childhood deaths. Respiratory disease is a major medical problem for persons with Down syndrome. These numbers reinforce the notion that a better understanding of why those with Down syndrome get more lung disease and congenital heart defects, but much less cardiovascular and atherosclerotic disease, will greatly benefit millions of people worldwide. Indeed, the study of outliers or phenotypic extremes in biology has yielded paradigm-shifting breakthroughs (*e.g.* thermophilic bacteria and thermostable enzymes for PCR [18] and the search for HIV-AIDS resistance genes [19]).

Respiratory disease in Down syndrome

Respiratory disease constitutes a large proportion of the morbidity in Down syndrome (table 1), and contributes to reduced life expectancy. Mortality rates for respiratory illnesses are significantly elevated in children [20] and in adults [21, 22] with Down syndrome. In Down syndrome, respiratory failure is a predictor of mortality [22]. There are limitations and potential biases in these studies, including data collection methods, lack of differentiation between primary diagnoses *versus* secondary diagnoses, and lack of long-term follow-up research. Nevertheless, respiratory disease contributes greatly to morbidity and mortality in Down syndrome.

Most of what is known about respiratory disease in Down syndrome comes from studies in the paediatric population. Studies in adults with Down syndrome with lung disease are sparse. Respiratory disease in Down syndrome can be organised into conditions affecting the upper airways, the lower airways and the pulmonary vasculature. The upper respiratory tract in persons with Down syndrome is often narrow due to congenital and associated conditions [23–25]. The trachea is often smaller in children with Down syndrome [26], and tracheal bronchus contributes to recurrent pneumonia [27, 28]. Airway malacia causes obstruction in >50% of children with Down syndrome, with other causes more prevalent in adults [29, 30]. These structural factors combine with hypotonia and obesity to increase the likelihood of proximal airway obstruction [31]. Sleep-related breathing disorders such as obstructive sleep apnoea occur in 39–79% of children with Down syndrome [32, 33]. In some patients with sleep apnoea, chronic intermittent hypoxia and respiratory acidosis contribute to pulmonary hypertension and cor pulmonale [34]. Infection of the upper airway, typically viral croup, is common in adults and children with Down syndrome. In a 20-year study of 239 children with Down syndrome, 23% presented with symptoms of stridor and 18% had persistent chronic croup [35].

Recurrent lower respiratory tract infection is common in persons with Down syndrome, especially children. Respiratory disease accounts for 43–78% of intensive care unit admissions, and 50% of those required ventilation support [12, 36, 37]. Respiratory illness is second only to CHD in mortality of individuals with Down syndrome at any age [38]. The average hospital stay due to lower respiratory illness is longer for children with Down syndrome [11]. One of the most important causes of lower respiratory tract infection is respiratory syncytial virus (RSV). Down syndrome is an independent risk factor for RSV bronchiolitis [36, 39]. Congenital abnormalities of the respiratory tract contribute not only to infection but also to chronic aspiration [37]. Delayed motor function and structural anomalies of the oral–nasal passages contribute to chronic aspiration manifested by persistent cough, wheezing and pneumonia [37]. Inherent deficiencies in innate and acquired immunity contribute to predisposition to respiratory tract infection. Poor response to vaccination potentially contributes to respiratory infection [40], as does decreased ciliary beat frequency, although ciliary ultrastructure in Down syndrome is normal [41].

Respiratory disease in Down syndrome can involve not only the airways but also the pulmonary vasculature. Pulmonary vascular diseases are wide-ranging in their aetiology and pathogenesis. Pulmonary embolism and pulmonary oedema are commonly found on computed tomography in pulmonary vascular diseases such as CHD, altitude sickness and pulmonary artery hypertension (PAH) [42–46]. Embolism and oedema in the lung secondary to left heart disease are thought to derive from changes in endothelial permeability and are probably related to potassium and calcium channel disturbances [47–49]. The lung vascular histopathology of persons with Down syndrome includes overall immaturity, double capillary networks and prominent intrapulmonary bronchopulmonary anastomoses [50–52]. In the fetal Down syndrome lung vasculature, overabundance of anti-angiogenic factors such as endostatin, collagen-4A3, amyloid protein precursor and tissue inhibitor of matrix metalloproteinase-3 may help to explain decreased vascularity and predisposition to pulmonary hypertension [53].

The high incidence of CHD in children with Down syndrome (discussed in detail later) is a major contributor to the incidence of group 1 PAH. In persons with Down syndrome and CHD with a left–right shunt, there is an imbalance in vasoactive mediators which favours vasoconstriction, platelet aggregation and cell proliferation in the pulmonary vasculature [54]. Infants with Down syndrome have a high rate of PAH that is disproportionate for their age [55–57], and ∼30% of adults with Down syndrome have septal defects and higher associated mortality compared to those without Down syndrome [58]. Persistent pulmonary hypertension of the newborn, another group 1 PAH disease, is quite prevalent in Down syndrome [59–61]. The combination of upper respiratory tract malformations, alveolar capillary dysplasia, hypoxia and hypercapnia may collectively promote development of pulmonary hypertension [62, 63]. Importantly, there have been no randomised controlled trials in Down syndrome with PAH examining response to vasodilator therapy [64, 65]. The available data suggest that persons with Down syndrome are equally as responsive as PAH patients without Down syndrome to endothelin receptor antagonists such as bosentan [64–68], although the 6-min walk test may not be an effective end-point [69]. Despite the high incidence of CHD in individuals with trisomy 21, pulmonary thrombosis does not appear to be a cause of morbidity or mortality in Down syndrome [70–74]. Children with Down syndrome are susceptible to rapid development of high-altitude pulmonary oedema, even at low altitudes (∼2000 m) [75]. Interestingly, the oedema was not secondary to left ventricular dysfunction. This may be due to increased pulmonary vasoreactivity and pulmonary vascular overperfusion and injury due to CHD [76] and/or due to pulmonary hypoplasia [51].

The lungs of persons with Down syndrome differ in structure and in terms of growth and development. These include enlarged alveolar airspaces, a generalised porous phenotype and subpleural cysts of undetermined significance [77]. Individuals with Down syndrome develop acute lung injury (ALI) at 10 times the rate of those without Down syndrome [14]. Since apoptosis of alveolar epithelial cells is a central feature of ALI [78, 79], it was though that increased apoptotic death would be observed in Down syndrome, but is not [80]. Although respiratory epithelial cells in Down syndrome are imbalanced with regard to free radical scavengers, exposure to oxidative stress does not result in increased apoptosis or inflammation [81]. From autopsy of persons with Down syndrome, it is known that the number of alveoli ranges from 58% to 83% predicted [52]. Such pulmonary hypoplasia is most obvious as small cysts in ∼20–36% of children with Down syndrome [82]. Thus, at some point beyond fetal life, apoptosis of alveolar epithelium may occur in persons with Down syndrome. Individuals with subpleural cysts are asymptomatic. Individuals with Down syndrome as well as mouse models of Down syndrome demonstrate reduced growth and a smaller body size [83]. Primary fibroblasts from individuals with Down syndrome proliferate at rates lower than control cells, and show increased susceptibility to apoptosis and senescence [84–87]. These observations, combined with reduced cerebellar size in Down syndrome [88, 89], suggest that reduced cell number is a general feature of Down syndrome. However, there are no data available that offer a direct comparison of rates of proliferation or apoptosis in lung cells from individuals with Down syndrome compared to controls. Moreover, age-related changes in rates of cell proliferation and/or apoptosis have not been determined in Down syndrome [90]. Thus, it is unclear whether arrested development or precocious ageing impacts respiratory infectious disease in Down syndrome.

Genetic influence of trisomy 21 on pulmonary disease

The Hsa21 gene pre-B-cell leukaemia homeobox-regulating protein-1 (PREP1) encodes a tumour suppressor transcription factor that plays important roles with regard to cell proliferation and epithelial to mesenchymal transition [91]. The p53 protein is a direct target of PREP1, and PREP1 is overexpressed in Down syndrome fibroblasts [92]. PREP1-overexpressing mice are smaller, as cells undergo increased rates of apoptosis due to overactivity of p53 [93]. Although no lung-specific data are available, p53 may also play a role in apoptosis resulting from overexpression of the Hsa21 gene Ets2 transcription factor [94]. Ets2 is a transcription factor and proto-oncogene that controls cell fate, proliferation and apoptosis [95]. Cells in Down syndrome harbour an imbalanced antioxidant defence system associated with high

expression of superoxide dismutase 1 (SOD1, Hsa21) [96]. Chronic oxidative stress in Down syndrome fibroblasts is accompanied by p21-dependent replicative senescence [97]. The balance of oxidants/antioxidants greatly affects the biology of the lung and is the subject of intense study [98]; however, there are no lung-specific data available regarding either SOD1 overexpression or antioxidant imbalance in the lungs of persons with Down syndrome. In addition, the Hsa21-encoded micro (mi)RNAs could contribute to hypocellularity. For example, high levels of the miRNA Let-7c induce cell cycle arrest by targeting CDC25a [99]. Similarly, the tumour suppressor activities of Hsa21-encoded miR-99a [100] and miR-125b-2 [101] could contribute to lung hypoplasia. The biology of the lung in Down syndrome can also be affected by overexpression of Hsa21 genes in nonresident cells. For example, lipopolysaccharide-induced ALI in mice is greatly promoted by macrophage expression of miR-155 [102] and miR-155 knockout mice are protected from chronic pulmonary fibrosis after bleomycin exposure [103].

Epigenetic modification of the genome in Down syndrome may also potentially explain dysregulation of lung development and homeostasis. In fibroblasts, the presence of an extra chromosome 21 is associated with hypermethylation of the embryonic organ morphogens HOXB (chromosome 17) and HOXD (chromosome 2) clusters [104]. The hypermethylation is due to upregulation of DNA methyltransferases DNMT3B (chromosome 20) and DNMT3L (Hsa21) and downregulation of demethylases TET2 (chromosome 4) and TET3 (chromosome 2). The epigenetic changes may help explain the existence of chromosomal domains of gene expression dysregulation (GEDDs) [105], and collectively, these data argue for global perturbation of the nuclear chromatin environment in Down syndrome. Intriguingly, the presence of GEDDs is conserved in the Ts65Dn mouse model of Down syndrome [105], even though the chromosomal context is different due to only partial Hsa21 synteny on mouse chromosome 16. In Down syndrome, hypermethylation of morphogenetic genes during development and during post-natal life may help to explain upper airway narrowing and obstruction, lower respiratory tract hypoplasia and inhibition of pulmonary angiogenesis.

With regard to the pulmonary vasculature in Down syndrome, the Hsa21 gene Down syndrome candidate region-1 (DSCR1/regulator of calcineurin (RCAN)-1) is overexpressed and encodes a negative regulator of vascular endothelial growth factor (VEGF)-calcineurin signalling [106, 107]. DYRK1A (Hsa21), also attenuates calcineurin signalling [106], and further angiogenic inhibition in Down syndrome could be provided by overexpression of Hsa21-encoded collagen XVIII, which can be cleaved into endostatin, a potent endogenous angiogenic inhibitor [108]. Non-Hsa21-encoded anti-angiogenesis factors are overexpressed in the fetal lung [53], and while collectively anti-angiogenesis contributes to low rates of solid tumour in Down syndrome [109], it may have negative implications for the pulmonary vasculature. For example, blockade of VEGF receptors in hypoxic rodents causes pulmonary hypertension [110].

Congenital heart defects and cardiovascular disease in Down syndrome

Down syndrome is associated with high incidence (45–50%) of CHD, especially atrioventricular septal defects (AVSD) (table 2) [4, 5]. Surgical correction has greatly decreased mortality in neonates with Down syndrome presenting with AVSD [111]. Uncorrected septal defects lead to shunting of systemic blood to the pulmonary circuit, increased blood flow and PAH, which may persist even after correction. There is an increased incidence of persistent pulmonary hypertension of the newborn (PPHN) in Down syndrome (~5.2% *versus* 0.1% in the general population) [59]. The pathophysiology and clinical management of PPHN have recently been reviewed [112]. Progress has been made with regard to biomarkers of paediatric PAH [113, 114], but not PAH in the setting of Down syndrome, (DS-PAH), and there are no animal models specific for DS-PAH. Many factors such as chronic hypoxia, sleep apnoea, recurrent respiratory infection, low birthweight and transient myeloproliferative disease probably contribute to DS-PAH or pulmonary hypertension in Down syndrome [63].

TABLE 2 Congenital heart disease and cardiovascular disease in individuals with Down syndrome

Children	Atrioventricular septal defects (these are the most common in children with Down syndrome)
	Ventricular septal defects
	Atrial septal defects
	Patent ductus arteriosus
	Tetralogy of Fallot
Adults	Acquired valvular heart disease, including mitral prolapse and valvular regurgitation
	Low rates of atherosclerosis, myocardial infarction and stroke
	Reduced rate of hypertension and less coronary artery intima medial thickness

Atherosclerotic disease is the leading cause of death in Europe and worldwide (~30% of all deaths worldwide) [115]. Death due to CAD is greater among females (51%) than males (42%), and ~20% of all deaths in Europe are due to coronary heart disease [115]. Morbidity associated with CAD, as measured by hospital discharge rates for CAD, is increasing [115]. Persons with Down syndrome have a low incidence of CAD, particularly atherosclerosis, in spite of increases in obesity and metabolic disturbances in Down syndrome [116]. Smoking is not a major risk factor for CAD in people with Down syndrome [10], and their circulating levels of cholesterol fractions may confer cardioprotection [6]. Atherosclerosis is a complex pathophysiological process involving inflammofibrotic remodelling and occlusion of systemic blood vessels, contributing greatly to the worldwide burden of CAD [117, 118]. Individuals with Down syndrome are resistant to development of atheroma and atherosclerosis [119–121]. A limitation of the earliest studies was that they were conducted using autopsy material from persons with Down syndrome who were institutionalised, where behavioural risks for CAD may have been reduced by control of dietary intake and levels of physical activity. A study found that the intimal media thickness of the carotid artery was lower in individuals with Down syndrome who did not reside in community housing, despite higher C-reactive protein, triglycerides and total body fat [122]. Interestingly, systolic and diastolic blood pressures are lower in Down syndrome subjects compared to controls [122]. In adults with Down syndrome who are aged ⩾30 years, hypertension and the use of anti-hypertensive drugs are lower than in the general population [123]. Hypertensive CAD is believed to affect ~1 billion people worldwide and is a major risk factor for stroke, myocardial infarction and kidney diseases [124]. Persons with Down syndrome may have different autonomic nervous system responses, which can affect cardiovascular function [125, 126].

Children and adults with Down syndrome commonly have hypothyroidism (prevalence ~25–60%, depending on the study [127–129]). Subclinical hypothyroidism in younger individuals is associated with increased risk of stroke [130]. Thyroid hormone levels are key homeostatic regulators of blood pressure and lipid levels, and have been correlated to heart failure and cardiovascular mortality.

Genetic influence of trisomy 21 on congenital heart disease

CHD in Down syndrome shows a "fixed pattern" of defects, with high numbers of septal defects, but low rates of transposition of the great vessels, tetralogy of Fallot or aortic coarctation [10]. Persons with Down syndrome appear to be protected against CAD despite elevated risk factors. How then does the presence of an extra chromosome 21 contribute to CHD yet protect against CAD? It must be pointed out that Down syndrome is not universally accompanied by CHD; thus, trisomy 21 itself is insufficient to cause CHD. The most recent data suggest that many Hsa21 (*i.e.* dosage-sensitive) genes are required for development of CHD, but that no single gene may be required [131, 132].

The COL-α1 (VI) and -α2 (VI) chains are encoded by genes located on Hsa21 and their overexpression has been associated with atrioventricular canal defects in Down syndrome [133, 134]. The α3 (VI) chain is encoded by the COL6A3 located at chromosome 2, and individuals with Down syndrome who have single nucleotide polymorphisms in COL6A3 are at increased risk of muscle hypotonia and CHD [135]. Loss-of-function mutations in the cell adhesion molecule cysteine-rich epidermal growth factor-like domain (CRELD)1, encoded on chromosome 3, contribute to CHD in Down syndrome [136]. Increased gene dosage of the Hsa21 gene junctional adhesion molecule (JAM)2 was recently shown to potentiate CHD in mice with CRELD1 mutations [137]. Similarly, haploinsufficiency of the heart morphogen Tbx5 (chromosome 12) results in different left–right cardiac patterning when on a trisomic background in Ts65Dn mice [138]. Collectively, these studies raise the possibility that overdosage of Hsa21 genes combined with perturbed expression of non-Hsa21 genetic modifiers may drive AVSD (and perhaps other developmental morbidities) in Down syndrome [139]. In this regard, many of the deleterious gene variants involving CHD identified to date involve the VEGF-A pathway [140].

As in the lung, Hsa21-encoded microRNAs may play a role in CHD in Down syndrome. In maternal peripheral blood, the plasma expression profile of fetal miR-let-7c and miR-99a are elevated in pregnancies with CHD-positive fetuses [141]. No published data are available evaluating maternal levels of Hsa21-encoded miRs with regard to CHD in Down syndrome. Let-7c controls lineage and stage-specific transcription factors that promote and direct cardiogenesis, while miR-99a has the opposite effects [142]. Thus, a balancing act of the levels of critical Hsa21-encoded miRs and/or proteins, rather than simply Hsa21 gene dosage, may ultimately govern the development of CAD. In another example, Hsa21-encoded PDE9a hydrolyses cGMP and is a major determinant of intracellular cGMP levels important for signalling cascades. PDE9a overexpression contributes to maladaptive hypertrophy and cardiac failure in humans and in a mouse model of aortic stenosis [143]. Conversely, expression of miR-99a correlates closely with cardiac function in mice, and overexpression of miR-99a attenuates both cardiomyocyte hypertrophy *in vitro* and aortic stenosis-associated cardiac hypertrophy *in vivo* [144].

As mentioned earlier, rates of CAD in persons with Down syndrome are very low relative to the general population. This is even more surprising given that adults with intellectual disability have higher incidences of cardiac disease, and individuals with Down syndrome commonly have sedentary lifestyles, poor diets, abnormalities in lipid metabolism and obesity [6, 7, 145, 146]. Plasma markers of sterol lipid metabolism (total cholesterol and lipoproteins) in Down syndrome are generally unchanged from age-matched controls and yield few clues into the reduced prevalence of atherosclerotic disease [147]. The question naturally arises: what Hsa21 genes are protective in Down syndrome and are these lower/disturbed in individuals who have CAD within the typical population? Decrease of plasma levels of homocysteine is a therapy that lowers risk of CAD and stroke [148]. Homocysteine is converted to cysteine by enzymatic action of cystionine-β-synthase (Hsa21). Cystionine-β-synthase is overexpressed in Down syndrome [149], and plasma levels of homocysteine are lower [150]. Further research involving persons with Down syndrome could more fully elucidate the mechanisms underlying the "homocysteine theory" of arteriosclerosis [121], a disease affecting a very large number of people worldwide.

The Hsa21-encoded RCAN1 inhibits the calcineurin-nuclear factors of activated T-cells (NFATc) signalling pathway [151]. RCAN1/DSCR1 may have a dual role with regard to the heart in Down syndrome. On one hand, proper developmental regulation of RCAN1 appears to be critical for proper regulation of valvuloseptal development [152]. On the other hand, RCAN1 expression is high in atherosclerotic lesions [153], and experimental inactivation of RCAN1 decreased atherosclerotic lesion burden [154]. This is paradoxical, given that atherosclerosis rates are low in Down syndrome despite overexpression of Hsa21 RCAN1. One clue might come from the suggestion that RCAN1 participates in a positive feedback loop involving inflammation, macrophages and oxidised low-density lipoproteins that potentiates lesion formation and progression [154]. There is evidence for oxidative and nitrosative stress in adults with Down syndrome [155, 156]. Additional clues for how RCAN1 could play a role in the development of atherosclerosis come from the setting of type II diabetes. Cardiovascular disease is the principal cause of death in persons with diabetes (~382 million people worldwide have diabetes), and reduction of atherosclerotic disease in diabetes is of major clinical importance [157]. RCAN1 is overexpressed in islet cells in type II diabetes without Down syndrome [158]. It is interesting that the Ts65Dn and Ts16 mouse models of Down syndrome are hyperglycaemic and show impaired glucose tolerance [158]. The incidence of type I diabetes is increased in Down syndrome [159], as is type II diabetes [160]. It may be that other overexpressed Hsa21-encoded genes in Down syndrome dampen RCAN1-influenced development of diabetes and atherosclerosis.

Hypertension affects ~1 billion people worldwide and is a risk factor for stroke and myocardial infarction [124]. The renin–angiotensin–aldosterone system is a hormone system that principally controls blood pressure [161]. Following conversion to angiotensin II from angiotensin I, angiotensin II receptors on blood vessels bind angiotensin II and vasoconstrict, thus increasing blood pressure. Angiotensin receptor blockers (sartans) are in widespread clinical use as antihypertensives [162]. Both females and males with Down syndrome have lower blood pressure than comparison subjects [163], and have lower intima media thickness of coronary arteries [122]. In a study on monozygotic twins discordant for Down syndrome, Hsa21-encoded miR-155 was found to translationally repress one allele of the type-1 angiotensin II receptor gene, resulting in reduced risk of hypertension [164].

Immune system disturbances impacting cardiopulmonary function in Down syndrome

Immune disturbances in Down syndrome account for an enormous and wide-ranging disease burden (table 3), especially pulmonary infectious disease. For persons with Down syndrome aged <3 years, respiratory illnesses are the most common cause of hospital admissions [165], and respiratory disease is by far the most common cause of death in persons with Down syndrome [38]. Both intrinsic immune defects

TABLE 3 Immune system disturbances in Down syndrome

Mild-to-moderate reduction in T-cell counts
Mild-to-moderate reduction in B-cell counts
Absence of normal lymphocyte expansion in infancy
Thymus size is smaller than age-matched controls
Mild-to-moderate reduction in naive T-cell percentages, with corresponding reduction of T-cell excision circles
Suboptimal antibody responses to immunisations
Decreased total and specific immunoglobulin A in saliva
Decreased neutrophil chemotaxis

and extrinsic (anatomical) factors contribute to disturbed immune function and respiratory infection in individuals with Down syndrome [166–168]. Reduced numbers of T- and B-lymphocytes and abnormalities of their function both undoubtedly contribute to altered immunoglobulin levels, poor responses to vaccinations and increased respiratory infections [36, 166, 169, 170]. In Down syndrome, the effects of an altered immune system on CHD or CAD are unknown.

In Down syndrome, the thymus is smaller than in control subjects and there are fewer mature T-cells expressing the αβ isoform of the T-cell receptor and CD3 [171]. In early childhood, T-lymphocytopenia is present in Down syndrome [172]. Predisposition to infection may continue into adulthood, even as T-lymphocytopenia wanes [172], because the T-lymphocyte phenotype and functional repertoire are abnormal [170]. A higher ratio of T-helper lymphocyte type 1 cells to T-helper lymphocyte type 2 cells and increased interferon-γ production are a feature of Down syndrome [173]. Intriguingly, severe RSV infection is hypothesised to result from disturbance in regulatory T-cell mediated control of host immune function [174].

Studies of B-lymphocytes in the Down syndrome population have revealed a complex portrait. Levels of immunoglobulin classes and responses to vaccination vary in Down syndrome [170]. In one study of 26 children with Down syndrome, only one individual had decreased IgG2 levels, yet 18 out of 26 had increased rates of infection [175, 176]. Following vaccination to influenza A or polysaccharide pneumococcus, antibody responses are active in persons with Down syndrome, yet antibody titres are lower [166, 169, 177, 178]. One possible contribution to increased prevalence and severity of pulmonary infection in Down syndrome could be reduced numbers of subsets of B-cells, such as switched memory B-cells [179, 180]. Deficit of such cells in Down syndrome could result in suboptimal protection from, and response to, infectious agents. Further evidence of B-cell dysfunction includes higher rates of autoimmunity in Down syndrome. In one study, 29% of children with Down syndrome showed positivity to at least one autoantibody, *versus* 8% in controls [167]. No correlation with infectious disease was investigated. The authors postulated that the presence of autoantibodies in Down syndrome reflected an early immune senescence in Down syndrome. There is currently no evidence that low T-cell (or B-cell) numbers correlate to incidence or severity of lung (or any) infection in Down syndrome [166], but the adaptive immune system in Down syndrome is intrinsically disturbed. Immunophenotyping and enumeration of peripheral blood leukocytes is an important tool to examine the immune system in Down syndrome. Data on resident lung and heart leukocytes are badly needed.

In Down syndrome, innate immune cell analyses show an erythroid cell-skewed developmental abnormality in haematopoiesis in fetal liver and yolk sac [181]. These findings are phenocopied to a large extent in mouse models of Down syndrome [182, 183]. In line with this, transient myeloproliferative disease and macrocytosis are commonly observed in Down syndrome [184]. Unfortunately, the putative role of abnormal numbers and/or function of myeloid-lineage cells in Down syndrome is unknown. Monocytes, macrophages, dendritic cells, neutrophils and natural killer cells play important roles in atherosclerotic plaque formation [185]. Since persons with Down syndrome are protected against atherosclerosis, the study of their innate immunology offers a unique opportunity to compare and contrast the repertoire of innate immune cells to the general population. Such studies may eventually lead to the development of new therapies that would aim to skew myeloid cell subpopulations towards more "atheroprotective" functionality. In the lung, the development and function of myeloid cell subsets is highly complex [186]. Nevertheless, comparative study of Down syndrome and control myeloid cells in the lung should yield insight into differences into the pathobiology of respiratory infection.

Genetic influence of trisomy 21 on immune system disturbances impacting cardiopulmonary function

Altered immunity in Down syndrome may greatly impact cardiopulmonary homeostasis. Several Hsa21-encoded genes may be of special importance in this regard. CD18 integrins are composed of a unique CD11 subunit noncovalently bound to CD18 (β_2 integrin, Hsa21). The role of these integrins is to support key leukocyte adhesive functions critical to antigen presentation, efferocytosis and pathogen clearance [187]. Some reports suggest that CD18 is overexpressed on Down syndrome myeloid cells [188]. However, other studies have not detected any increased CD18 on myeloid cells [189], and the differential findings may be related to whether the cells used had been transformed (lymphoblastoid cells). Due to the importance of CD18 in mediating appropriate leukocyte responses [187], the potential correlation of CD18 expression to gene dosage in Down syndrome should be examined carefully.

The Hsa21 gene Sumo3 was identified in a screen of differentially methylated genes in Down syndrome. Sumo3 was methylated compared to controls, but paradoxically, gene expression for Sumo3 was increased 1.5-fold in accordance with trisomic gene dosage [190]. Sumo3 functions in post-translational sumoylation of proteins regulating immunoglobulin production by B-cells [191] and cytokine production in T-cells

[192]. In addition, tight regulation of Sumo proteins has been shown to be critical for cardiac development, cardiac metabolism and cardiac contractility [193]. In another gene methylation screen in Down syndrome, RUNX1 was identified [194]. RUNX1 is a transcription factor that regulates haematopoiesis [195] and is implicated in leukaemia in Down syndrome [196]. In addition, this study highlighted an epigenetic signature in Down syndrome that affects expression of genes involved in cell adhesion molecules, autoimmune thyroid disease, type I diabetes and PI3k-Akt signalling.

Recently, the expression of 20 inflammation-related genes (non-Hsa21) were analysed in peripheral blood obtained from individuals with Down syndrome. Leukocytes from children with Down syndrome expressed less bradykinin receptor B1, which the authors hypothesised might compromise a number of cytokine production pathways and lead to a higher frequency of lung infection [197]. Collectively, the DNA methylation and gene expression studies corroborate the notion that there is a global gene expression change in the genome (Hsa21 and non-Hsa21) [197]. This signature appears to be preserved in inducible pluripotent stem cell (iPSC)-derived progenitors in Down syndrome [181, 198]. Use of iPSCs will be critical tools in the comparative study of developmental and tissue-specific stages in haematopoietic development in Down syndrome and in the typical population.

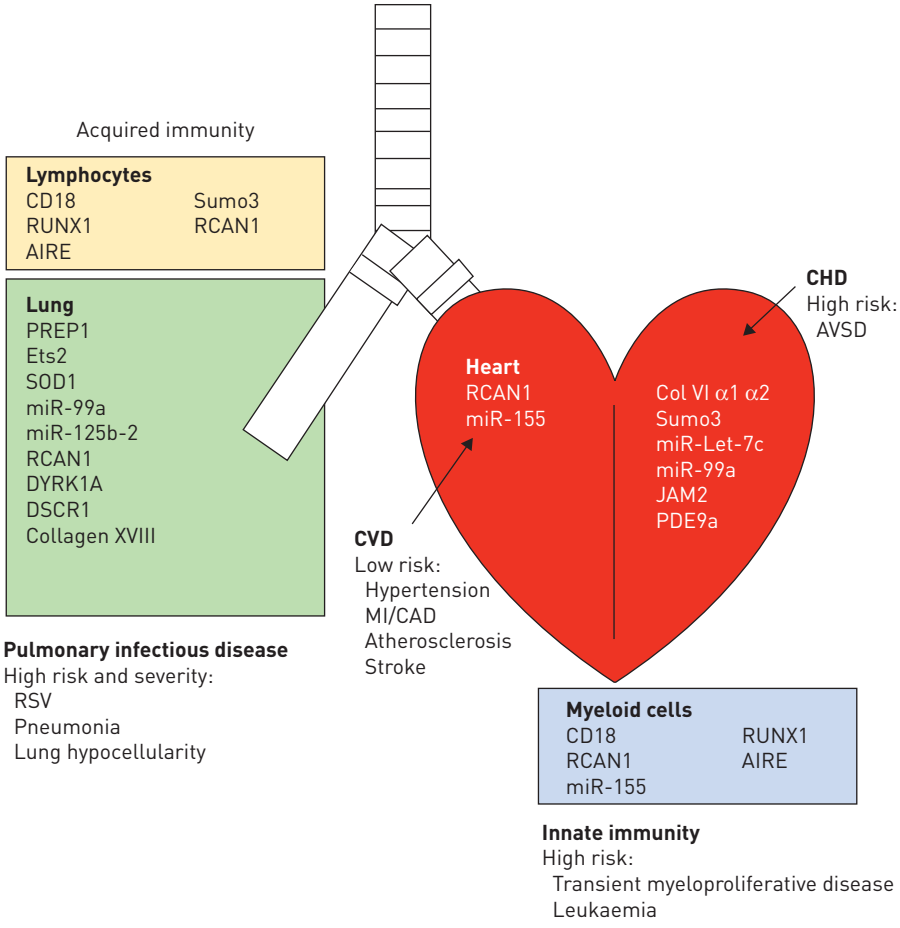

FIGURE 2 Research and cardiopulmonary disease in Down syndrome: opportunities for therapeutic leverage. A number of Hsa21-encoded genes affect organ homeostasis. Persons with Down syndrome have low rates of cardiovascular disease (CVD), despite elevated risk factors. In contrast, congenital heart disease (CHD) is highly prevalent in Down syndrome. Pulmonary infectious disease is the leading cause of mortality in Down syndrome, caused by both intrinsic (morphological factors) and extrinsic (immune dysfunction) factors. Listed in each organ cartoon are genes implicated in disturbed heart, lung and immune function. Research into the mechanisms of resistance to development of coronary artery disease and solid tumours inherent in persons with Down syndrome will undoubtedly benefit the larger population (*i.e.* therapeutic leverage). MI/CAD: myocardial infarction/coronary artery disease; AVSD: atrioventricular septal defect; RSV: respiratory syncytial virus.

As mentioned earlier, Down syndrome is associated with increased risk and severity of viral and bacterial pneumonias. RCAN1, encoded on Hsa21, regulates inflammatory responses to *Pseudomonas* infection both *in vitro* and *in vivo* [199]. Interestingly, dysregulation of NFAT, NF-κB and STAT3 signalling pathways was observed in the setting of RCAN1 deficiency. Specifically, although RCAN1 deficiency led to increased bacterial clearance, the mice still died due to systemic inflammation. The authors suggest that overexpression of RCAN1 in Down syndrome may alter downstream effector signalling in pathological ways. In support of this, reduced interleukin-10 and increased STAT3 pathway activation has been reported in Down syndrome [200, 201].

Persons with Down syndrome are prone to develop autoimmune dysfunction. Some aspects of the immmunophenotypic constellation seen in Down syndrome are evocative of autoimmune–polyendocrinopathy–candiasis–ectodermal–dystrophy (APECED), a disease characterised by depressed immune function and autoimmunity. APECED is caused by inactivating mutations in the autoimmune regulator (AIRE) transcription factor gene, which resides on Hsa21 [202]. Decreased AIRE protein expression results in an altered programme of downstream gene expression that compromises myeloid immune cell numbers, phenotypes and function [203]. During development, AIRE controls expression of peripheral tissue specific antigens in medullary thymic epithelial cells through which selection of T-cell clones is accomplished [204]. Autoantibodies and mutations in AIRE have been described in Down syndrome with autoimmune polyendocrine syndrome type I [205], leading to thymic hypofunction and primary immunodeficiency [206]. These studies establish connections between trisomy 21, reduced AIRE and loss of central tolerance. Insufficiency of AIRE may also contribute to the interferonopathy observed in Down syndrome [207], since APECED patients have expanded memory T-cell subsets that produce interferon-γ [202]. AIRE expression has been reported to be decreased in Down syndrome [105].

Conclusions and specific recommendations

With regard to biomedical research and Down syndrome, there is good reason for optimism as we look to the future. However, several limiting factors should be highlighted. We lack solid demographic data with regard to incidence and prevalence of Down syndrome worldwide. Further complicating the study of Down syndrome is the difficulty to ascertain the effect of healthcare systems used by persons with Down syndrome and their families. Individuals with Down syndrome experience similar health conditions and thus use similar services, but they more often experience multiple conditions [22]. We assert that improving the lives of persons with Down syndrome through a renaissance of Hsa21-focused basic and translational research will, in turn, improve the lives of persons without Down syndrome worldwide (figure 2). To reach this goal (faster), we propose the following action points. First, the biomedical research community should increase efforts to reach out to the Down syndrome population and their families to participate in research studies (and *vice versa*). Second, we need to increase the health-related demographic data available regarding persons with Down syndrome. Third, we should increase funding for basic research focused on cardiopulmonary disease and immunity in Down syndrome, in addition to the important ongoing and future cognitive studies. In the United States, Down syndrome is the most common chromosomal abnormality among live-born infants, yet unfortunately it receives the lowest funding for any genetic condition. Admittedly, the current climate of funding worldwide is extremely challenging. Increased funding is likely to be expedited by increased awareness of the huge impact of cardiopulmonary disease on persons with Down syndrome, and the high potential to leverage research findings to improve the health of all.

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