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/tIGU306iMKG

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

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

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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 inflammobifibrotic 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 COLα1 (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 α2 (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 hydrolysates 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 |

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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 through 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 (β2 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.
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 immunophenotypic constellation seen in Down syndrome are evocative of autoimmune–polyendocrinopathy–candidiasis–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.

References

1. Weijerman ME, van Furth AM, Vonk Noordegraaf A, et al. Prevalence, neonatal characteristics, and first-year mortality of Down syndrome: a national study. *J Pediatr* 2008; 152: 13–19.
2. Roizen NJ, Patterson D. Down’s syndrome. *Lancet* 2003; 361: 1281–1289.
3. Frid C, Drott P, Lundell B, et al. Mortality in Down’s syndrome in relation to congenital malformations. *J Intellect Disabil Res* 1999; 43: 234–241.
4. Am Acad Pediatr. Committee on Genetics. American Academy of Pediatrics: health supervision for children with Down syndrome. *Pediatrics* 2001; 107: 442–449.
5. Freeman SB, Bean LH, Allen EG, et al. Ethnicity, sex, and the incidence of congenital heart defects: a report from the National Down Syndrome Project. *Genet Med* 2008; 10: 173–180.
6. Dörner K, Gaethle AS, Tolksdorf M, et al. Cholesterol fractions and triglycerides in children and adults with Down’s syndrome. *Clin Chim Acta* 1984; 142: 307–311.
7. Melville CA, Cooper SA, McGrother CW, et al. Obesity in adults with Down syndrome: a case–control study. *J Intellect Disabil Res* 2005; 49: 125–133.
8. Baird PA, Sadovnick AD. Causes of death to age 30 in Down syndrome. *Am J Hum Genet* 1988; 43: 239–248.
9. Licastro F, Marocchi A, Penco S, et al. Does Down’s syndrome support the homocysteine theory of atherogenesis? Experience in elderly subjects with trisomy 21. *Arch Gerontol Geriatr* 2006; 43: 381–387.
10. Vis JC, Duffels MG, Winter MM, et al. Down syndrome: a cardiovascular perspective. *J Intellect Disabil Res* 2009; 53: 419–425.
51 Cooney TP, Thurlbeck WM. Pulmonary hypoplasia in Down's syndrome. N Engl J Med 1982; 307: 1170–1173.
52 Schloo BL, Vawter GF, Reid LM. Down syndrome: patterns of disturbed lung growth. Hum Pathol 1991; 22: 919–923.
53 Galambos C, Minic AD, Bush D, et al. Increased lung expression of anti-angiogenic factors in down syndrome: potential role in abnormal lung vascular growth and the risk for pulmonary hypertension. PLos One 2016; 11: e0159005.
54 Fukushima H, Kosaki K, Sato R, et al. Mol quasi-apoptotic factors in Down syndrome. J Pediatr 1990; 32: 60–66.
55 Greenwood RD, Nadas AS. The clinical course of cardiac disease in Down’s syndrome. Pediatrics 1976; 58: 893–897.
56 Rodriguez FH 3rd, Moodie DS, Parekh DR, et al. Outcomes of hospitalization in adults in the United States with atrial septal defect, ventricular septal defect, and atrioventricular septal defect. Am J Cardiol 2011; 108: 290–293.
57 Weijerina ME, van Furth AM, van der Mooren MD, et al. Prevalence of congenital heart defects and persistent pulmonary hypertension of the neonate with Down syndrome. Eur J Pediatr 2010; 169: 1195–1199.
58 Duffels MG, Vis JC, van Loon RL, et al. Down patients with Eisenmenger syndrome: is bosentan treatment an option? Int J Cardiol 2009; 134: 378–383.
59 Kermeen FD, Franks C, O’Brien K, et al. Endothelin receptor antagonists are an effective long term treatment option in pulmonary arterial hypertension associated with congenital heart disease with or without trisomy 21. Heart Lung Circ 2010; 19: 326–337.
60 Shah PS, Hellmann J, Adatia I. Clinical characteristics and follow up of Down syndrome infants without congenital heart disease who presented with persistent pulmonary hypertension of newborn. J Perinat Med 2004; 32: 168–170.
61 Cua CL, Blankenship A, North AL, et al. Increased incidence of idiopathic persistent pulmonary hypertension in Down syndrome neonates. Pediatr Cardiol 2007; 28: 250–254.
62 Hasegawa N, Oshima M, Kawakami H, et al. Effect of oxidative stress on respiratory epithelium from children with Down syndrome. J Perinat Med 2011; 39: 591–596.
63 Duffels MG, Vis JC, van Loon RL, et al. Effect of bosentan on exercise capacity and quality of life in adults with pulmonary arterial hypertension associated with congenital heart disease with and without Down’s syndrome. Am J Cardiol 2009; 103: 1309–1315.
64 Bicopterz FA Jr, Melvin WS, Basilius D, et al. Congenital heart disease in Down’s syndrome patients: a decade of surgical experience. Thorax Cardiovasc Surg 1989; 37: 369–371.
65 Kaya MG, Lam YY, Eber B, et al. Long-term effect of bosentan therapy on cardiac function and symptomatic benefits in adult patients with Eisenmenger syndrome. Is Card Fail 2012; 18: 379–384.
66 D’Alto M, Romeo E, Argiento P, et al. Therapy for pulmonary arterial hypertension due to congenital heart disease and Down’s syndrome. Int J Cardiol 2013; 164: 323–326.
67 Mohan UR, Mangat JS, Sutaria N, et al. Saddle arterial embolus in a patient with Down syndrome. Pediatr Cardiol 2006; 27: 117–121.
68 Grikaitis M, Ang C. Acute-altitude induced hypoxia in a child with Down’s syndrome following postoperative repair of complete atrioventricular septal defect. BMJ Case Rep 2011; 2011: bcr0220113866.
69 Biko DM, Schwartz M, Anupindi SA, et al. Subpleural lung cysts in Down syndrome: prevalence and association with coexisting diagnoses. Pediatr Radiol 2008; 38: 280–284.
70 Matute-Bello G, Martin TR. Science review: apoptosis in acute lung injury. Crit Care 2003; 7: 355–358.
71 Martin TR, Nakamura M, Matute-Bello G. The role of apoptosis in acute lung injury. Crit Care Med 2003; 31: S184–S188.
72 Spoor M, de Thuisen JH, van der Loos CM, et al. Pulmonary epithelial apoptosis in fetal Down syndrome: not higher than normal. Pediatr Dev Pathol 2012; 15: 199–205.
73 Gonzalez OR, Gomez IG, Recalde AL, et al. Postnatal development of the cystic lung lesion of Down syndrome: suggestion that the cause is reduced formation of peripheral air spaces. Pediatr Pathol 1991; 11: 623–633.
74 Roper RJ, St John HK, Philip J, et al. Perinatal loss of T66Dn Down syndrome mice. Genetics 2006; 172: 437–443.
75 Segal DJ, McCoy EE. Studies on Down’s syndrome in tissue culture. I. Growth rates and protein contents of fibroblast cultures. J Cell Physiol 1974; 83: 85–90.
120 Murdoch JC, Rodger JC, Rao SS, Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis.

117 Nichols M, Townsend N, Scarborough P, Colvin KL, Yeager ME. Proteomics of pulmonary hypertension: could personalized profiles lead to personalized medicine?

112 Jain A, McNamara PJ. Persistent pulmonary hypertension of the newborn: advances in diagnosis and treatment. Semin Fetal Neonatal Med 2015; 20: 262–271.

108 van Gameren-Oosterom HB, van Dommelen P, Schonbeck Y, Taraseviciene-Stewart L, Kasahara Y, Alger L, Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down syndrome.

113 J Thorac Cardiovasc Surg 2008; 135: 230.

114 van Gemen-Oosterom HB, van Dommelen P, Schonbeck Y, et al. DNA-methylation patterns in trisomy 21 using cells from mononzygotic twins. PLoS One 2015; 10: e0135555.

115 Letourneau A, Santoni FA, Bonilla X, et al. Domains of genome-wide gene expression dysregulation in Down's syndrome. Nature 2014; 508: 345–350.

109 de Haan JB, Susil B, Pritchard M, et al. An altered antioxidant balance occurs in Down syndrome fetal organs: implications for the "gene dosage effect" hypothesis. J Neurotransm Suppl 2003; 67: 67–83.

116 Contestabile A, Fil A, Cappellini A, et al. Widespread impairment of cell proliferation in the neonate Ts65Dn mouse, a model for Down syndrome. Cell Prolif 2009; 42: 171–183.

111 Rasekino M, Manda N, Iavarone F, et al. Transcription factor PREP1 induces EMT and metastasis by controlling the TGF-β-SMAD3 pathway in non-small cell lung adenocarcinoma. Proc Natl Acad Sci USA 2014; 111: E3775–E3784.

107 Saffirio C, Marino B, Formigari R. Better surgical prognosis for patients with Down syndrome. J Thorac Cardiovasc Surg 2008; 135: 230.

108 Gutierrez, W, Lindeman G, Nicholls D, et al. Evaluation of the utility of microarray-based gene expression analysis in Down's syndrome. Cell Prolif 2005; 38: 359–363.

109 Service RK, Burnham CA, Bernier WR, et al. SNP arrays identify CNVs in a sporadic case of Down syndrome and in monozygotic twins.

108 Service RK, Burnham CA, Bernier WR, et al. SNP arrays identify CNVs in a sporadic case of Down syndrome and in monozygotic twins. J Cell Biochem 2004; 91: 896–903.

107 Camacho-Catalán M, Pascual-Soler A, et al. Down syndrome: a new pathology for large arteries? Cerebrovasc Dis 2004; 18: 113–119.

106 Rodriguez-Sureda V, Vilches A, Sánchez O, et al. Intracellular oxidant activity, antioxidant enzyme defense system, and cell senescence in fibroblasts with trisomy 21. Oxid Med Cell Longev 2015; 2015: 509241.

105 Dani C, Poggi C. The role of genetic polymorphisms in antioxidant enzymes and potential antioxidant therapies in neonatal lung disease. Antioxid Redox Signal 2014; 21: 1863–1880.

104 Sailani MR, Santoni FA, Letourneau A, et al. DNA-methylation patterns in trisomy 21 using cells from mononzygotic twins. PLoS One 2015; 10: e0135555.

103 Liao J, Chen H, et al. Inhibition of the VEGF receptor 2 combined with chronic inhibition of the VEGF receptor 2 attenuates the role of the calcineurin inhibitor DSCR1. Cell Prolif 2009; 42: 171–183.
Serrano-Candelas E, Farré D, Aranguren-Ibáñnez A, Méndez-Barbero N, Esteban V, Villahoz S, Agiovlasitis S, Collier SR, Baynard T, Lim SS, Vos T, Flaxman AD, Campos C, Guzmán R, López-Fernández E, Pogribna M, Melnyk S, Pogribny I, Sharav T, Collins RM Jr, Baab PJ. Growth studies in infants and children with Down syndrome.

Brattström L, Englund E, Brun A. Does Down syndrome support homocysteine theory of arteriosclerosis? Lancet 1987; 1: 391–392.

Draheim CC, Geijer JR, Dengel DR. Comparison of intima-media thickness of the carotid artery and cardiovascular disease risk factors in adults with versus without the Down syndrome. Am J Cardiol 2010; 106: 156–1512.

Alexander M, Petri H, Ding Y, et al. Morbidity and medication in a large population of individuals with Down syndrome compared to the general population. Dev Med Child Neurol 2016; 58: 246–254.

Lim SS, Vos T, Flaxman AD. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2224–2260.

Agiovlasitis S, Baynard T, Pitetti KH, et al. Heart rate complexity in response to upright tilt in persons with Down syndrome. Res Dev Disabil 2011; 32: 2102–2107.

Agiovlasitis S, Collier SR, Baynard T, et al. Autonomic response to upright tilt in people with and without Down syndrome. Res Dev Disabil 2010; 31: 857–863.

Sharav T, Collins RM Jr, Baab PJ. Growth studies in infants and children with Down’s syndrome and elevated levels of thyrotropin. Am J Dis Child 1988; 142: 1302–1306.

Tüysüz B, Beker DB. Thyroid dysfunction in children with Down’s syndrome. Acta Paediatr 2001; 90: 1389–1393.

King K, O’Gorman C, Gallagher S. Thyroid dysfunction in children with Down syndrome: a literature review. Ir J Med Sci 2014; 183: 1–6.

Chaker L, Baumgartner C, den Elzen WP, et al. Subclinal hypothyroidism and the risk of stroke events and fatal stroke: an individual participant data analysis. J Clin Endocrinol Metab 2015; 100: 2181–2191.

Lana-Eloa E, Watson-Scales S, Slender A, et al. Genetic dissection of Down syndrome-associated congenital heart defects using a new mouse mapping panel. Elife 2016; 5: e11614.

Ramachandran D, Zeng Z, Locke AE, et al. Genome-wide association study of Down syndrome-associated atrioventricular septal defects. G3 2015; 5: 1961–1971.

Davies GE, Howard CM, Farrer MJ, et al. Genetic variation in the COL6A1 region is associated with congenital heart defects in trisomy 21 (Down’s syndrome). Ann Hum Genet 1995; 59: 253–269.

Baptista MJ, Fairbrother UL, Howard CM, et al. Heterotrasomy, a significant contributing factor to ventricular septal defect associated with Down syndrome? Hum Genet 2000; 107: 476–482.

Dey A, Bhowmik C, Chatterjee A, et al. Down syndrome related muscle hypotonia: association with COL6A3 functional SNP rs2270669. Front Genet 2013; 4: 57.

Rupp PA, Fouad GT, Egelston CA, et al. Identification, genomic organization and mRNA expression of CRELD1, the founding member of a unique family of matricellular proteins. Gene 2002; 293: 47–57.

Li H, Edie S, Klinedinst D, et al. Penetration of congenital heart disease in a mouse model of Down syndrome depends on a trisomic potentiator of a disomic modifier. Genetics 2016; 203: 763–770.

Polk RC, Gergics P, Steimle JD, et al. The pattern of congenital heart defects arising from reduced Tbx5 expression is altered in a Down syndrome mouse model. BMC Dev Biol 2015; 15: 30.

Li H, Cherry S, Klinedinst D, et al. Genetic modifiers predisposing to congenital heart disease in the sensitized Down syndrome population. Circ Cardiovasc Genet 2012; 5: 301–308.

Ackerman C, Locke AE, Feingold E, et al. An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. Am J Hum Genet 2012; 91: 646–659.

Kehler L, Biro O, Lazar L, et al. Elevated hsa-miR-99a levels in maternal plasma may indicate congenital heart defects. Biomed Rep 2015; 3: 869–873.

Coppola A, Remito A, Borel C, et al. Cardiomyogenesis is controlled by the miR-99a/let-7c cluster and epigenetic modifications. Stem Cell Res 2014; 12: 323–337.

Lee DI, Zhu G, Sasaki T, et al. Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. Nature 2015; 519: 472–476.

Li Q, Xie J, Wang B, et al. Overexpression of microRNA-99a attenuates cardiac hypertrophy. PLoS One 2016; 11: e0148480.

Beange H, McElduff A, Baker W. Medical disorders of adults with mental retardation: a population study. Am J Ment Retard 1995; 99: 595–604.

van den Akker M, Maaskant MA, van der Meijden RI. Cardiac diseases in people with intellectual disability. J Intellect Disabil Res 2006; 50: 515–522.

Tansley G, Holmes DT, Lüthojann D, et al. Sterol lipid metabolism in Down syndrome revisited: Down syndrome is associated with a selective reduction in serum brassicasterol levels. Curr Gerontol Geriatr Res 2012; 179138.

Antoniades C, Antonopoulos AS, Tousoulis D, et al. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J 2009; 30: 6–15.

Ait Yahya-Graison E, Aubert J, Dauphinot L, et al. Classification of human chromosome 21 gene expression variations in Down syndrome: impact on disease phenotypes. Am J Hum Genet 2007; 81: 475–491.

Pogribna M, Melnyk S, Pogribny I, et al. Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet 2001; 69: 88–95.

Serrano-Candelas E, Farré D, Aranguren-Ibañez A, et al. The vertebrate RCAN gene family: novel insights into evolution, structure and regulation. PLoS One 2014; 9: e85539.

Lange AW, Molkentin JD, Yutzey KE. DSCR1 gene expression is dependent on NFATc1 during cardiac valve formation and colocalizes with anomalous organ development in trisomy 16 mice. Dev Biol 2004; 266: 346–360.

Toric E, Gaman L, Atanasiu V. The regulator of calcineurin (RCAN1) an important factor involved in atherosclerosis and cardiovascular diseases development. J Med Life 2014; 7: 481–487.

Méndez-Barbero N, Esteban V, Villahoz S, et al. A major role for RCAN1 in atherosclerosis progression. EMBO Mol Med 2013; 5: 1901–1917.

Campos C, Guzmán R, López-Fernández E, et al. Evaluation of urinary biomarkers of oxidative/nitrosative stress in adolescents and adults with Down syndrome. Biochim Biophys Acta 2011; 1812: 760–768.
156 Muchová J, Žižňárová I, Duračková Z. Oxidative stress and Down syndrome. Do antioxidants play a role in therapy? Physiol Res 2014; 63: 535–542.
157 Low Wang CC, Hess CN, Hiatt WR, et al. Clinical update: cardiovascular disease in diabetes mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus – mechanisms, management, and clinical considerations. Circulation 2016; 133: 2459–2502.
158 Peiris H, Duffield MD, Facista J, et al. A syntenic cross species aneuploidy genetic screen links RCAN1 expression to β-cell mitochondrial dysfunction in type 2 diabetes. PLoS Genet 2016; 12: e1006033.
159 Bergholdt R, Eising S, Nerup J, et al. Increased prevalence of Down’s syndrome in individuals with type 1 diabetes in Denmark: a nationwide population-based study. Diabetologia 2006; 49: 1179–1182.
160 Taggart L, Coates V, Truesdale-Kennedy M. Management and quality indicators of diabetes mellitus in people with intellectual disabilities. J Intellect Disabil Res 2013; 57: 1152–1163.
161 Strauss MH, Hall AS. The divergent cardiovascular effects of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on myocardial infarction and death. Prog Cardiovasc Dis 2016; 58: 473–482.
162 Te Riet L, van Esch JE, Roks AJ, et al. Hypertension: renin-angiotensin-aldosterone system alterations. Circ Res 2015; 116: 960–975.
163 Draheim CC, McCubbin JA, Williams DP. Differences in cardiovascular disease risk between nondiabetic adults with mental retardation with and without Down syndrome. Am J Ment Retard 2002; 107: 201–211.
164 Sethupathy P, Borel C, Gagnebin M, et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3′ untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. Am J Hum Genet 2007; 81: 405–413.
165 So SA, Urbano RC, Hodapp RM. Hospitalizations of infants and young children with Down syndrome: evidence from inpatient person-records from a statewide administrative database. J Intellect Disabil Res 2007; 51: 1030–1038.
166 Ram G, Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol 2011; 164: 9–16.
167 da Rosa Utiyama SR, Nisihara RM, Nass FR, et al. Autoantibodies in patients with Down syndrome: early senescence of the immune system or precocious markers for immunological diseases? Paediatr Child Health 2008; 44: 182–186.
168 Verstegen RH, Kusters MA, Gemen EF, et al. Down syndrome B-lymphocyte subpopulations, intrinsic defect or decreased T-lymphocyte help. Pediatr Res 2010; 67: 563–569.
169 Costa-Carvalho BT, Martinez RM, Dias AT, et al. Antibody response to pneumococcal capsular polysaccharide vaccine in Down syndrome patients. Braz J Med Biol Res 2006; 39: 1587–1592.
170 Kusters MA, Verstegen RH, Gemen EF, et al. Intrinsic defect of the immune system in children with Down syndrome: a review. Clin Exp Immunol 2009; 156: 189–193.
171 Murphy M, Epstein LB. Down syndrome (trisomy 21) thymuses have a decreased proportion of cells expressing high levels of TCR alpha, beta and CD3. A possible mechanism for diminished T cell function in Down syndrome. Clin Immunol Immunopathol 1990; 55: 453–467.
172 de Hingh YC, van der Vossen PW, Gemen EF, et al. Intrinsic abnormalities of lymphocyte counts in children with Down syndrome. J Pediatr 2005; 147: 744–747.
173 Franciotta D, Verri A, Zardini E, et al. Interferon-γ and interleukin-4-producing T cells in Down’s syndrome. Neurosci Lett 2006; 395: 67–70.
174 Christiaansen AF, Syed MA, Ten Eyck PP, et al. Altered Treg and cytokine responses in RSV-infected infants. Pediatr Res 2016; 80: 702–709.
175 Loh RK, Harth SC, Thong YH, et al. Immunoglobulin G subclass deficiency and predisposition to infection in Down’s syndrome. Pediatr Infect Dis J 1990; 9: 547–551.
176 Loh RK, Thong YH, Ferrante A. Immunoglobulin G subclass deficiency in children with high levels of immunoglobulin E and infection proneness. Int Arch Allergy Appl Immunol 1990; 93: 285–288.
177 Epstein LB, Philip R. Abnormalities of the immune response to influenza antigen in Down syndrome (trisomy 21). Prog Clin Biol Res 1987; 246: 163–182.
178 Epstein CJ, Weil J, Epstein LB. Abnormalities in the interferon response and immune systems in Down syndrome: studies in human trisomy 21 and mouse trisomy 16. Prog Clin Biol Res 1987; 246: 191–208.
179 Carsetti R, Valenti D, Marcellini V, et al. Reduced numbers of switched memory B cells with high terminal differentiation potential in Down syndrome. Eur J Immunol 2015; 45: 903–914.
180 Valentini D, Marcellini V, Bianchi S, et al. Generation of switched memory B cells in response to vaccination in Down syndrome children and their siblings. Vaccine 2015; 33: 6689–6696.
181 Maclean GA, Menne TF, Guo G, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci USA 2012; 109: 17567–17572.
182 Carmichael CL, Majewski J, Alexander WS, et al. Hematopoietic defects in the Ts1Cje mouse model of Down syndrome. Blood 2009; 113: 1929–1937.
183 Kirschammer G, Ilanis S, Liu H, et al. Highly penetrant myeloproliferative disease in the Ts65Dn mouse model of Down syndrome. Blood 2008; 111: 767–775.
184 Chou ST, Opalinska JB, Yao Y, et al. Trisomy 21 enhances human fetal erythro-megakaryocyte development. Blood 2008; 112: 4503–4506.
185 Chávez-Sánchez L, Espinosa-Luna JE, Chávez-Rueda K, et al. Innate immune system cells in atherosclerosis. Arch Med Res 2014; 45: 1–14.
186 Kopf M, Schneider C, Nobs SP. The development and function of lung-resident macrophages and dendritic cells. Nat Immunol 2015; 16: 36–44.
187 Rosetti F, Mayadas TN. The many faces of Mac-1 in autoimmune disease. Immunol Rev 2016; 269: 175–193.
188 Taylor GM, Williams A, D’Souza SW, et al. The expression of CD18 is increased on trisomy 21 (Down syndrome) lymphoblastoid cells. Clin Exp Immunol 1988; 71: 324–328.
189 Barrena MJ, Echaniz P, Garcia-Serrano C, et al. Differential expression of lymphocyte function-associated antigen (LFA-1) on peripheral blood leucocytes from individuals with Down’s syndrome. Clin Exp Immunol 1992; 88: 41–44.
190 Kerkl K, Schupf N, Hatta K, et al. Altered DNA methylation in leukocytes with trisomy 21. PLoS Genet 2010; 6: e10001212.
Dobreva G, Dambacher J, Grosschedl R. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin μ gene expression. *Genes Dev* 2003; 17: 3048–3061.

Terui Y, Saad N, Jia S, *et al.* Dual role of sumoylation in the nuclear localization and transcriptional activation of NFAT1. *J Biol Chem* 2004; 279: 28257–28265.

Mendlar L, Braun T, Müller S. The ubiquitin-like SUMO system and heart function: from development to disease. *Circ Res* 2016; 118: 132–144.

Bacalini MG, Gentilini D, Boattini A, *et al.* Identification of a DNA methylation signature in blood cells from persons with Down syndrome. *Aging* 2015; 7: 82–96.

Fonatsch C. The role of chromosome 21 in hematology and oncology. *Genes Chromosomes Cancer* 2010; 49: 497–508.

Nižetić D, Groet J. Tumorigenesis in Down’s syndrome: big lessons from a small chromosome. *Nat Rev Cancer* 2012; 12: 721–732.

Silva CR, Biselli-Périco JM, Zampieri BL, *et al.* Differential expression of inflammation-related genes in children with Down syndrome. *Mediators Inflamm* 2016; 2016: 6985903.

Chou ST, Byrskas-Bishop M, Tober JM, *et al.* Trisomy 21-associated defects in human primitive hematopoiesis revealed through induced pluripotent stem cells. *Proc Natl Acad Sci USA* 2012; 109: 17573–17578.

Junkins RD, MacNeil AJ, Wu Z, *et al.* Regulator of calcineurin 1 suppresses inflammation during respiratory tract infections. *J Immunol* 2013; 190: 5178–5186.

Tanaka MH, Giro EM, Cavalcante LB, *et al.* Expression of interferon-γ, interferon-α and related genes in individuals with Down syndrome and periodontitis. *Cytokine* 2012; 60: 875–881.

Cavalcante LB, Tanaka MH, Pires JR, *et al.* Expression of the interleukin-10 signaling pathway genes in individuals with Down syndrome and periodontitis. *J Periodontol* 2012; 83: 926–935.

Heikkilä N, Laakso SM, Mannerström H, *et al.* Expanded CD4+ effector/memory T cell subset in APECED produces predominantly interferon gamma. *J Clin Immunol* 2016; 36: 555–563.

Anderson MS, Su MA. AIRE expands: new roles in immune tolerance and beyond. *Nat Rev Immunol* 2016; 16: 247–258.

Taniguchi RT, DeVoss JJ, Moon JI, *et al.* Detection of an autoreactive T-cell population within the polyclonal repertoire that undergoes distinct autoimmune regulator (Aire)-mediated selection. *Proc Natl Acad Sci USA* 2012; 109: 7847–7852.

Söderbergh A, Gustafsson J, Ekwall O, *et al.* Autoantibodies linked to autoimmune polyendocrine syndrome type I are prevalent in Down syndrome. *Acta Paediatr* 2006; 95: 1657–1660.

Lima FA, Moreira-Filho CA, Ramos PL, *et al.* Decreased AIRE expression and global thymic hypofunction in Down syndrome. *J Immunol* 2011; 187: 3422–3430.

Sullivan KD, Lewis HC, Hill AA, *et al.* Trisomy 21 consistently activates the interferon response. *Elife* 2016; 5: e16220.