ARTICLES

NFAT dysregulation by increased dosage of *DSCR1* and *DYRK1A* on chromosome 21

Joseph R. Arron[1]*, Monte M. Winslow[2]*, Alberto Polleri[1]*, Ching-Pin Chang[3], Hai Wu[1], Xin Gao[1], Joel R. Neilson[2], Lei Chen[1], Jeremy J. Heit[4], Seung K. Kim[4], Nobuyuki Yamasaki[7], Tsuyoshi Miyakawa[7], Uta Francke[5], Isabella A. Graef[1]* & Gerald R. Crabtree[1,4,6]

Trisomy 21 results in Down's syndrome, but little is known about how a 1.5-fold increase in gene dosage produces the pleiotropic phenotypes of Down's syndrome. Here we report that two genes, *DSCR1* and *DYRK1A*, lie within the critical region of human chromosome 21 and act synergistically to prevent nuclear occupancy of NFATc transcription factors, which are regulators of vertebrate development. We use mathematical modelling to predict that autoregulation within the pathway accentuates the effects of trisomy of *DSCR1* and *DYRK1A*, leading to failure to activate NFATc target genes under specific conditions. Our observations of calcineurin- and *Nfatc*-deficient mice, *Dscr1*- and *Dyrk1a*-overexpressing mice, mouse models of Down's syndrome and human trisomy 21 are consistent with these predictions. We suggest that the 1.5-fold increase in dosage of *DSCR1* and *DYRK1A* cooperatively destabilizes a regulatory circuit, leading to reduced NFATc activity and many of the features of Down's syndrome. More generally, these observations suggest that the destabilization of regulatory circuits can underlie human disease.

Genetic regulatory circuits evolve to be well-suited for their biological tasks and to be robust under the varying conditions encountered over the course of development and during responses to environmental stimuli[1,2]. However, the operation of feedback loops, buffers and amplifiers within these circuits could also make them susceptible to conditions other than those that drove their evolution. One such condition would be chromosomal trisomy, the best-known example of which is Down's syndrome. The many features of Down's syndrome include neurological, skeletal, cardiovascular and immunological defects, and are generally thought to originate from a 1.5-fold increase in the dosage of genes within a critical region of chromosome 21, which is present in triplicate in all cases of Down's syndrome[3–6]. In general, chromosomal trisomy is lethal, suggesting that the buffering mechanisms of many genetic regulatory circuits are susceptible to variations in gene dosage.

When studying the developmental roles of calcineurin and NFAT signalling, one of the authors (I.A.G.) noted striking similarities between the phenotypic features of Down's syndrome and mice carrying deletions of genes encoding components of the NFAT signalling pathway. This pathway, which is a critical regulator of vertebrate development and organogenesis[7,8], is initiated by Ca^{2+} entry and results in calcineurin activation. Calcineurin dephosphorylates NFATc proteins, leading to their nuclear entry and assembly with partner proteins (NFATn) to form NFAT transcription complexes[9]. Rephosphorylation by an unknown priming kinase and glycogen synthase kinase 3 (GSK3) exports NFATc proteins from the nucleus[8,10,11] (see overview in Supplementary Fig. 1).

Phenotypes of *Nfatc*-null mice

Specific facial features are characteristic of Down's syndrome and arise from changes in embryonic bone development[6,12,13]. *Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$ double-knockout mice have significantly reduced length between the intersection of the parietal and interparietal bones and the nasale, a narrowed gap between the anterior aspects of the zygomatic arches, and shortened anterior parts of the skull (Fig. 1a–c and Supplementary Figs 2, 3). These animals display significantly shortened distances between the inferior-most point on the alveolar rim at the bone–tooth junction and both the mandibular angle and the posterior-most point on the mandibular condyle, but distances between posterior mandibular landmarks are less shortened (Supplementary Fig. 4). These characteristics of *Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$ mice resemble those observed in human Down's syndrome.

Individuals with Down's syndrome have cognitive deficits and muscular hypotonia, and often have sociable personalities[6,12,14]. Previous work has shown that mice with forebrain-specific deletion of the protein phosphatase calcineurin B1 (*Cnb1*, also known as *Ppp3r1*), the activity of which is regulated by DSCR1, have defects in learning and memory[15]. In addition, calcineurin/NFAT signalling is essential for axonal outgrowth in response to neurotrophins and netrins[16] during embryogenesis, and NFATc4 is a survival factor for cerebellar granule cells[17], a cell population that is decreased in Down's syndrome individuals[12] and mouse models[18]. NFAT signalling also has an established role in myogenesis[8,19], and we found that certain interneuron subpopulations fail to develop in *Nfatc2*$^{-/-}$; *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$ triple-knockout mice (H.W., I.A.G. and G.R.C., submitted manuscript). These findings suggest that even minor impairments in NFAT signalling might be sufficient to produce cognitive, behavioural and neuromuscular defects. In addition, we find that *Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$ mice show increased social interaction, increased locomotor activity, decreased muscular strength and decreased anxiety-related behaviour relative to control mice (Fig. 1d–g, Supplementary Fig. 5g–i). These characteristics are similar to those observed in Down's syndrome[12,14].

[1]Department of Pathology, [2]Program in Immunology, [3]Division of Cardiovascular Medicine, Department of Medicine, [4]Department of Developmental Biology, [5]Department of Genetics and [6]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California 94305, USA. [7]Genetic Engineering and Functional Genomics Unit, HMRO, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan.
*These authors contributed equally to this work.

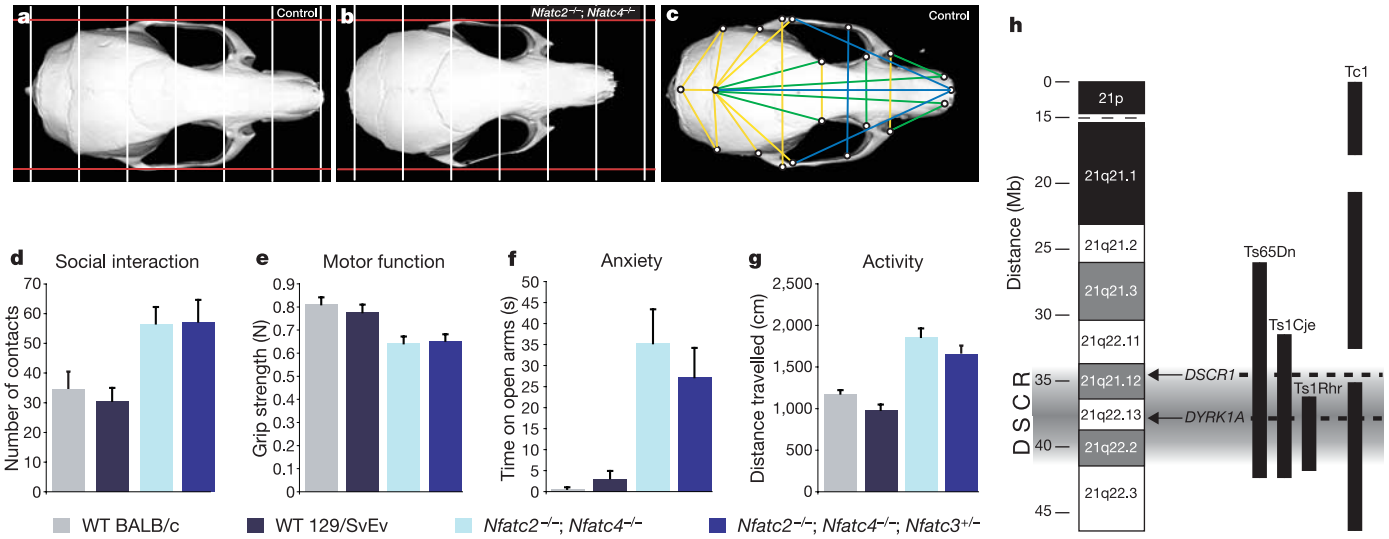

Figure 1 | Down's syndrome phenotypes in mice with mutations in the NFAT pathway. **a–c**, Superior view of crania from control (**a**, **c**) and *Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$ double-knockout (**b**) mice. **d**, NFATc mutant mice showed increased social interactions compared to BALB/c ($P = 0.0036$) and 129/SvEv ($P = 0.0008$) controls. **e**, Grip strength of NFATc mutants was significantly weaker ($P < 0.0001$ versus BALB/c, $P = 0.0009$ versus 129/SvEv). **f**, NFATc mutants entered significantly more into the open arms of an elevated plus maze and spent more time in open arms ($P < 0.0001$ versus BALB/c, $P < 0.0001$ versus 129/SvEv). **g**, NFATc mutants also showed an increase in locomotor activity ($P < 0.0001$ versus BALB/c, $P < 0.0001$ versus 129/SvEv). Error bars in **d–g** indicate s.e.m. **h**, Map of human chromosome 21q. The DSCR is indicated in shaded area. Bars on the right denote the extent of the conserved orthologous region of MMU16 or transchromosomic HSA21 triplicated in Down's syndrome model mice.

Table 1 summarizes additional studies that we have conducted and compares features of Down's syndrome with the phenotypes of NFATc mutants and mouse models of Down's syndrome. In the present study, we find that NFATc mutant mice manifest the following characteristic features of Down's syndrome: placental vascular abnormalities (leading to the death of most fetuses with Down's syndrome; Supplementary Fig. 5a, b), increased sociability (Fig. 1d and Supplementary Fig. 5g), hypotonia (Fig. 1e and Supplementary Fig. 5h), an annular pancreas (Supplementary Fig. 5c, d) and an aganglionic megacolon (Supplementary Fig. 5e, f). We and others have shown that NFATc mutant mice manifest vascular and cardiac morphogenic defects[20,21], delayed tooth eruption (M.M.W. and G.R.C., submitted manuscript), behavioural changes (Fig. 1), a tendency to diabetes (J.J.H. and S.K.K., submitted manuscript), obstructive nephropathy[22], muscular weakness[8,19] and immunodeficiencies[7,8,23]. Segmental trisomy 16 mice with genomic triplications orthologous to parts of human chromosome 21 (HSA21) have been described. Certain features of Down's syndrome such as placental insufficiency, cardiac defects, genitourinary abnormalities and gastrointestinal malformations are not observed in segmental trisomic mice, but are present in NFATc mutant mice. Although no individual NFATc mutant mouse reproduces all Down's syndrome pathologies, the features of Down's syndrome seem to be mild forms of NFATc mutant phenotypes.

The Down's syndrome critical region

These observations led us to examine the human Down's syndrome critical region (DSCR; see Supplementary Discussion A) for genes

Table 1 | Summary of Down's syndrome-like phenotypes in NFATc mutant and partial trisomy 16 mice

Phenotype	Down's syndrome individuals	NFATc mutant mice	Ts65Dn[26]	Ts1Cje[27]	Ts1Rhr[30]	Tc1[29]
Placenta	Fetal loss (31–54%)	*Cnb1*$^{-/-}$ *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$[20]*	No	No	No	No
Cardiovascular	Endocardial cushion defects (65%)[12,14]	*Cnb1*$^{-/-}$ *Nfatc1*$^{-/-}$ *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$ *Nfatc2*$^{-/-}$; *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$ CsA treatment[20,21]	No	No	No	Minimal
Neurological	Cognitive defects, hypotonia, increased sociability[12]	*Cnb1*$^{-/-}$ *Nfatc2*$^{-/-}$; *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$ *Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$†* CsA treatment[15,16]	Yes[6]	Yes[6]	NR	Yes
Gastrointestinal	11% (1.4% Hirschsprung disease, 1.4% annular pancreas)[12,14]	*Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$*	No	No	NR	NR
Skeletal	Brachycephaly, midface hypoplasia, delayed tooth eruption[12]	*Nfatc2*$^{-/-}$; *Nfatc4*$^{-/-}$† *Nfatc1*$^{-/-}$‡	Yes[13] NR	Yes[28] NR	No NR	Minimal NR
Immune	Decreased interleukin-2 production and T-cell proliferation[12]	*Nfatc1*$^{-/-}$ *Nfatc3*$^{-/-}$ *Nfatc1*$^{-/-}$; *Nfatc2*$^{-/-}$ CsA treatment[7,8,23]	NR	NR	NR	Minimal
Genitourinary	Obstructive nephropathy (18%)[12,14]	*Cnb1*$^{-/-}$[22]	NR	NR	NR	NR

CsA, cyclosporin A; NR, not recorded.
*See Supplementary Fig. 5.
†See Fig. 1 and Supplementary Figs 2–4.
‡M.M.W. and G.R.C., submitted manuscript.

that might inhibit NFATc function. *DSCR1* is located at the centromeric border of the DSCR (Fig. 1h) and encodes an inhibitor of calcineurin/NFAT signalling[24,25]. *Dscr1* is triplicated in Ts65Dn and Ts1Cje mice, which have Down's syndrome-like craniofacial defects, but not in Ts1Rhr mice or Tc1 mice, which lack such craniofacial defects[12,13,26–30] (see Fig. 1h for an overview of the trisomic regions in Tn65Dn, Ts1Cje, Ts1Rhr and Tc1 mice). *DSCR1* is also expressed at higher levels in Down's syndrome fetuses[25].

We examined the 25–30 genes in the DSCR for other potential NFAT regulators and identified *DYRK1A*, which encodes a nuclear serine/threonine kinase[31] (Figs 1h and 2) that primes substrates for phosphorylation by GSK3. GSK3 phosphorylates NFATc proteins in the nucleus, resulting in their inactivation and export[10,11]. *DYRK1A* is expressed at elevated levels in some human Down's syndrome fetal tissues. *Dyrk1a*-deficient mice have defects in central nervous system (CNS) development[32], and overexpression produces neurodevelopmental defects[33]. *Dyrk1a* is sensitive to gene dosage, as heterozygous mutant mice show changes in CNS development[32].

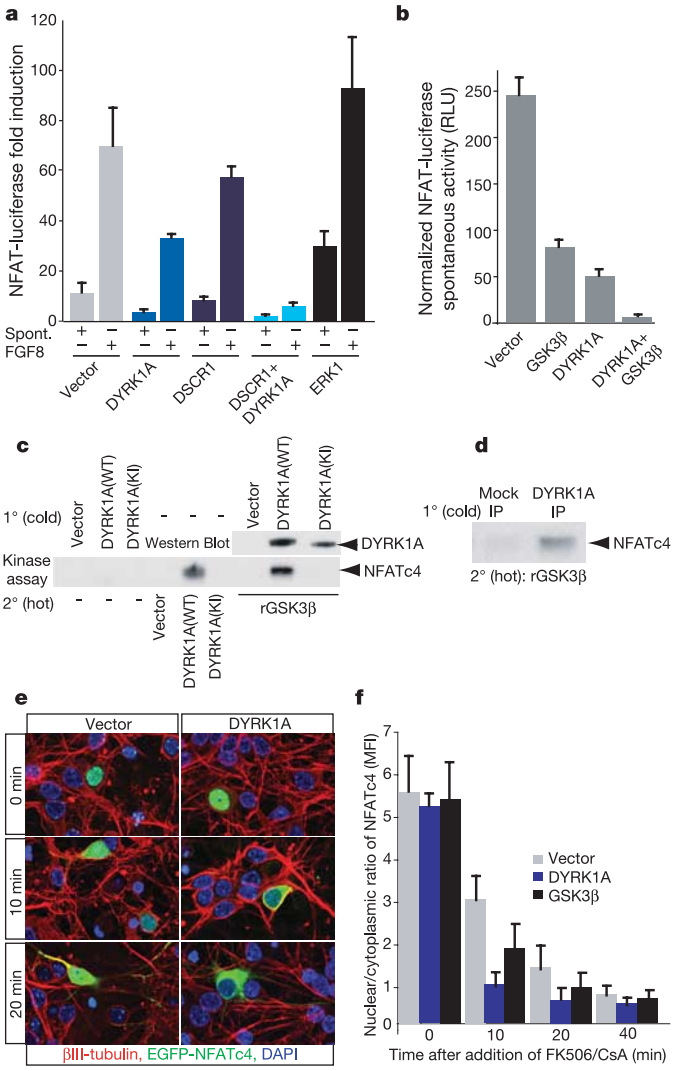

Figure 2 | DYRK1A is a nuclear export kinase for NFATc4. a, DYRK1A and DSCR1 synergistically block NFAT-dependent transcription in cortical neurons. Expression of DYRK1A and/or DSCR1 inhibits the activation of NFAT-dependent transcription in response to spontaneous activity (spont.) or stimulation by FGF8. **b**, DYRK1A and GSK3β synergistically inhibit NFAT-dependent transcription in cortical neurons. **c**, Wild-type DYRK1A, but not a kinase-inactive mutant DYRK1A(KI), phosphorylates NFATc4 and primes it for phosphorylation by GSK3β. 1° (cold), unlabelled ATP; 2° (hot), γ-^{32}P ATP. **d**, Endogenous DYRK1A immunoprecipitated from nuclear extracts can prime NFATc4 for GSK3β phosphorylation. **e**, Accelerated nuclear export of an NFATc4–enhanced green fluorescent protein (EGFP) fusion protein after overexpression of DYRK1A, shown in representative confocal sections. Nuclear export was initiated by the addition of FK506/cyclosporin A (CsA) at $t = 0$ min. Original magnification, 40×. **f**, Quantification of NFATc4–EGFP cytoplasmic and nuclear mean fluorescence intensity (MFI) 0, 10, 20 and 40 min after the addition of FK506/CsA. Error bars indicate s.d.

We found that DYRK1A regulates calcineurin/NFAT signalling in response to fibroblast growth factor-8 (FGF8), which has critical roles in development (Fig. 2a). DYRK1A, but not ERK1, inhibits FGF8-mediated induction of NFAT activity. Moreover, DYRK1A synergizes with DSCR1 to block NFAT-dependent transcription (Fig. 2a). We also investigated the role of DYRK1A in embryonic neurons responding to spontaneous Ca^{2+} channel activity, which is required for neural development[34,35]. Spontaneous activity induces a 12-fold increase in NFAT activity, which is blocked by DYRK1A (Fig. 2a). The role of DYRK1A in antagonizing NFAT may be general, in that NFAT-dependent transcription in HEK-293T cells is sensitive to DYRK1A and DSCR1 (Supplementary Fig. 6).

GSK3 is required for transcriptional inactivation and export of NFATc proteins[10,11]. It targets the first serine of a SPxxSP motif only if the second serine has been previously phosphorylated by a priming kinase. We found that DYRK1A synergizes with GSK3 to inhibit NFAT-dependent transcription in cortical neurons (Fig. 2b). Furthermore, DYRK1A, but not a kinase-inactive mutant (DYRK1A(KI)) can phosphorylate NFATc4 and prime it for subsequent phosphorylation by GSK3 (Fig. 2c). Endogenous DYRK1A immunoprecipitated from bFGF-stimulated H19-7 cells can prime NFATc4 for phosphorylation by GSK3 (Fig. 2d). Two conserved motifs in the amino termini of NFATc proteins, the serine-rich region and the serine/proline repeats, are the major sites of phosphorylation. Serine-to-alanine mutation of critical serines in these regions renders NFATc proteins independent of calcineurin activity and constitutively nuclear[10]. Using serine-to-alanine mutants of the serine-rich region and serine/proline repeats of NFATc4 together with mass spectrometric analysis (Supplementary Fig. 7), we found that DYRK1A phosphorylates the serine/proline repeats of NFATc4, consistent with a role as a priming kinase for GSK3[36].

NFATc proteins are rapidly exported from the nucleus, allowing discrimination between brief and sustained Ca^{2+} signals[37]. We found that DYRK1A-transfected neurons show more than threefold faster rates of NFATc4 export than those transfected with an empty vector, and almost twofold faster export rates than those transfected with GSK3 (Fig. 2e, f). Thus DYRK1A directs nuclear export of NFATc4. DYRK1A, but not DYRK1A(KI), prevents nuclear occupancy by NFATc1 (Supplementary Fig. 6) in HEK-293T cells. Thus, we conclude that DYRK1A reduces NFAT transcriptional activity by direct phosphorylation of NFATc proteins, which leads to their nuclear export.

Consequences of increasing *Dscr1* and *Dyrk1a* dosage

To determine whether increasing the dosage of *Dscr1* and *Dyrk1a* can reproduce features of Down's syndrome, we generated nine lines of double transgenic mice overexpressing *Dyrk1a* and *Dscr1* during embryonic development, and studied those with low levels of DYRK1A and DSCR1 protein expression. We monitored cardiac defects, which occur in half of Down's syndrome individuals[12] and all *Nfatc2*$^{-/-}$; *Nfatc3*$^{-/-}$; *Nfatc4*$^{-/-}$ triple-knockout[21] and *Nfatc1*$^{-/-}$ embryos[21,38,39]. Overexpression of DYRK1A (Fig. 3a, lane 2) at levels that are 2–3-fold above the endogenous protein is sufficient to induce vascular defects and block heart valve development (Fig. 3b). This modest overexpression of DYRK1A alone also decreases endogenous DSCR1and NFATc4 protein levels (Fig. 3a, lane 2), indicating that DYRK1A can inhibit expression of NFAT target genes, as predicted for an NFAT export kinase. Expression of both DYRK1A and DSCR1 to 1.5–2-fold above endogenous levels (Fig. 3a, lane 3) leads to failure of heart valve elongation at embryonic day (E)13.5 (arrow in Fig. 3d),

comparable to that observed in *Nfatc1*$^{-/-}$ embryos[21,38,39]. Moreover, endocardially expressed NFATc1 is hyperphosphorylated and localized to the cytoplasm of these *Dyrk1a*/*Dscr1*-overexpressing animals (Fig. 3e–g).

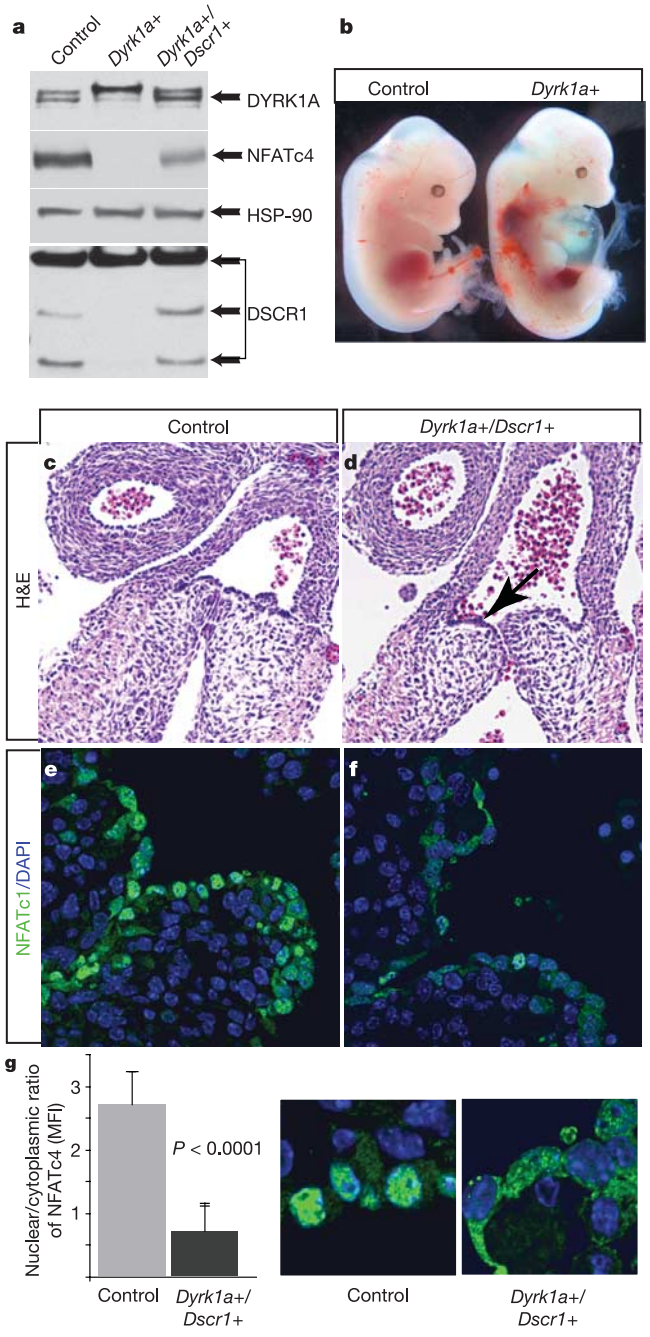

Figure 3 | Transgenic overexpression of *Dyrk1a* and *Dscr1* produces defects similar to NFAT mutants and Down's syndrome individuals. **a**, Immunoblots of control (lane 1) and *Dyrk1a*-overexpressing (lane 2, *Dyrk1a*+) and *Dyrk1a*/*Dscr1*-overexpressing transgenic (Lane 3, *Dyrk1a*+/*Dscr*+) E13.5 embyros. **b**, Gross morphology of an E13.5 control embryo (left) and a transgenic embryo transiently overexpressing *Dyrk1a* (right) with enlarged pericardial sac, vascular anomalies and heart failure. **c**, **d**, Blunted heart valves (arrow) in E13.5 *Dyrk1a*/*Dscr1*-overexpressing embryos. Original magnification, 20×. **e**, **f**, Confocal images showing redistribution of NFATc1 from the nucleus to the cytoplasm of endocardial cells in *Dyrk1a*/*Dscr1* double transgenic embryos. Original magnification, 40×. **g**, Quantification of the reduction in nuclear occupancy in *Dyrk1a*/*Dscr1* double transgenic embryos. Error bars indicate s.d. Immunofluorescent images are higher magnification insets of **e** and **f**.

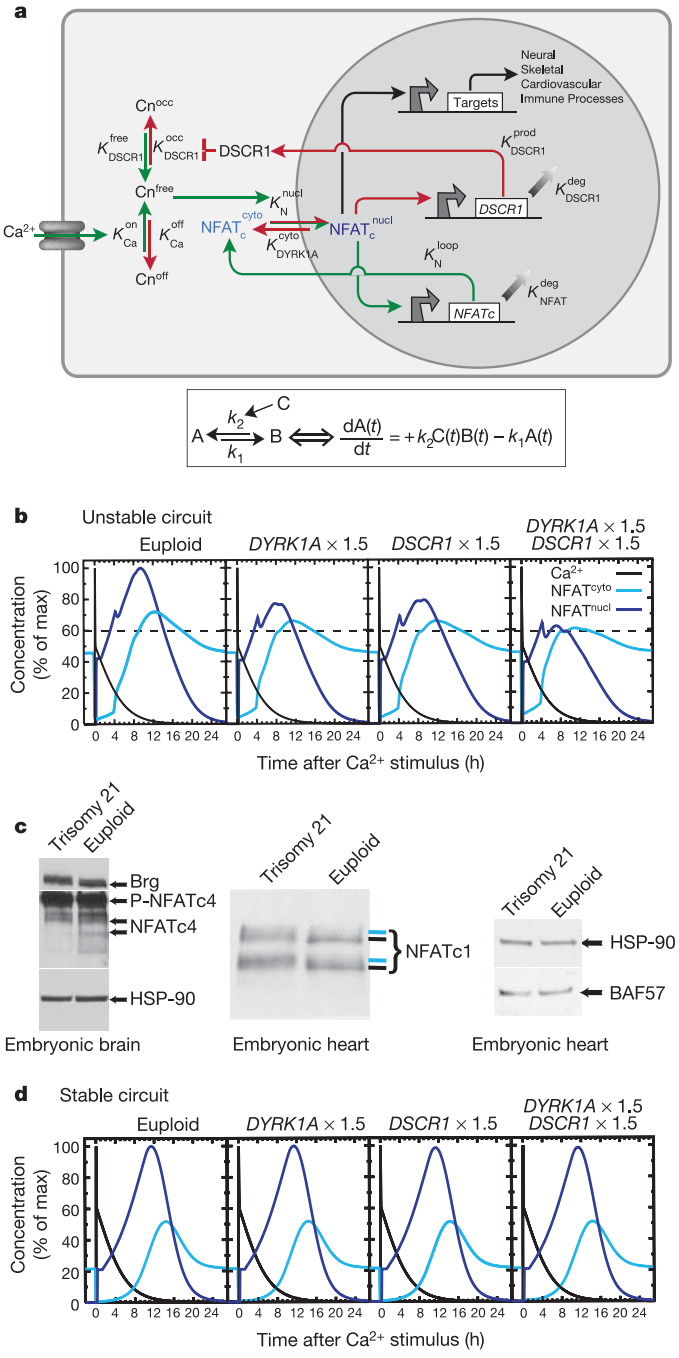

Figure 4 | Increased dosage of *DYRK1A* and *DSCR1* can significantly destabilize the NFAT regulatory circuit. **a**, The NFAT genetic regulatory circuit. Steps that increase the output of the genetic regulatory circuit are shown in green and steps that decrease the output of the circuit are in red. A generalized equation at the bottom is used as a template to calculate the change in concentration of each component over time. cyto, cytoplasmic (inactive) NFATc; deg, degradation; loop, positive or negative feedback loop; nucl, nuclear (active) NFATc; occ, occupied by DSCR1 (inactive enzyme); prod, production (gene transcription and translation). **b**, Simulation of NFAT regulatory circuit output under conditions of varying gene dosage of *DYRK1A* and *DSCR1* for an 'unstable circuit' in which nuclear NFATc levels are sensitive to a 1.5-fold increase in *DYRK1A* and *DSCR1* levels. Theoretical thresholds for gene activation are depicted at 60% of maximum. **c**, Hyperphosphorylated NFATc4 (P-NFATc4) and NFATc1 in 20-week trisomic human fetal brain and heart, relative to age-matched euploid tissues. **d**, Model of NFAT regulatory circuit output for a 'stable circuit' in which nuclear NFATc levels are relatively immune to 1.5-fold increases in *DYRK1A* and *DSCR1* levels.

We have found that NFATc4 positively regulates its own promoter through conserved NFAT binding sites in its regulatory region (Supplementary Fig. 8a, b). Positive feedback regulation is also consistent with the reduction in NFATc4 levels in *Dyrk1a*/*Dscr1*-overexpressing mice (Fig. 3a, lanes 2 and 3) and in *Cnb1*$^{-/-}$ mice (Supplementary Fig. 8c). These results indicate that NFATc4, like NFATc1[40], regulates its own promoter (Supplementary Fig. 8d). These positive feedback loops should enhance the effects of a 1.5-fold increase in *DSCR1* and *DYRK1A* gene dosage in human Down's syndrome.

Mathematical modelling of the NFAT genetic regulatory circuit

These feedback buffering and amplification mechanisms, intrinsic to NFAT signalling, led us to use mathematical modelling to better predict the output of NFAT signalling (Fig. 4a). Because the initial level of each of the proteins involved in NFAT signalling is developmentally regulated in the tissues involved in Down's syndrome, we used experimentally determined ranges of starting conditions rather than a single level (Fig. 4a and Supplementary Discussion B). The initial Ca^{2+} signal was treated as a continuous profile to mimic spontaneous activity, which is a major regulator of both neural development[34,35] and NFAT activity[11] (Fig. 2a). Two generalizations emerge. First, DSCR1 and DYRK1A have additive effects under most initial concentrations of each component. Second, if we assume, as other studies have demonstrated[41], that activating the promoters of target genes requires a threshold level of NFAT complexes, these genes may fail to be transcribed when *DSCR1* and/or *DYRK1A* are increased by 1.5-fold (Fig. 4b). Thus we predict that trisomy 21 will produce a disproportionate reduction in NFAT activity, resulting in a mild version of the aggregate defects observed in NFATc mutant mice.

To determine whether these predictions are borne out in mouse models of Down's syndrome, we examined two models with segmental trisomy[26,27]. In cortical neurons of E13.5 Ts1Cje embryos, we found an increase in DYRK1A expression levels and also increased phosphorylation of NFATc4 (Supplementary Fig. 9). However, in whole heads of E11.5 Ts1Cje embryos and in hippocampal neurons of postnatal day (P)1 Ts65Dn mice we observed neither increased DYRK1A or DSCR1 protein levels nor hyperphosphorylation of NFATc4 (Supplementary Fig. 9 and data not shown).

We also examined tissues from three human Down's syndrome fetuses and three age-matched controls at 17–21 weeks of gestation. During embryonic development, calcineurin/NFAT signalling is only required for brief and sharply defined time windows, and reduction in the activity of the pathway during these windows will phenocopy certain aspects of calcineurin/*Nfat*-null mutations[16,20,21]. Although many NFAT-dependent developmental programmes have been executed by this stage, we still observed hyperphosphorylated forms of NFATc4 and NFATc1 in the brain and heart, respectively, of one 20-week trisomic fetus relative to control tissues (Fig. 4c). This finding is consistent with calcineurin inhibition by DSCR1 and/or phosphorylation of NFATc by DYRK1A. Consistent with our model, which predicts that the NFAT genetic regulatory circuit may be unstable in some tissues (Fig. 4b, c) but stable in others (Fig. 4d), we found that NFATc1 phosphorylation was unchanged in spleen and muscle from the same fetus (not shown). These findings indicate that developmentally defined conditions are likely to lead to either stable or unstable states of the NFAT genetic regulatory circuit.

Discussion

Our studies suggest that under certain conditions, and perhaps for only brief developmental periods, increased dosage of *DSCR1* and *DYRK1A* in Down's syndrome reduces NFAT transcriptional activity, giving rise to mild versions of NFATc mutant phenotypes (Supplementary Fig. 1). Although certain post-developmental Down's syndrome pathologies, such as acute megakaryoblastic leukaemia and early-onset Alzheimer's disease, result from triplication of other genes on HSA21 (*RUNX1* and *APP*, respectively), perturbation of the NFAT genetic regulatory circuit by increased dosage of *DSCR1* and *DYRK1A* may explain many of the developmental phenotypes in Down's syndrome. Here we were able to study only a limited number of trisomic tissues at a developmental stage that was perhaps past the critical period for NFAT function in development[16,20,21]. Thus, additional work with human trisomic tissues from earlier developmental periods will be required to confirm our predictions.

Certain common but variable features of Down's Syndrome, such as heart disease, lethal placental vascular defects and immunodeficiency, are not seen in mouse models with segmental trisomy of murine chromosomal regions corresponding to HSA21[12,26,27]. The absence of cardiovascular defects and immunodeficiency in the mouse models might reflect small differences in the kinetic constants between humans and mice shown in Fig. 4a. Subtle differences in these constants can be amplified in the positive and negative feedback loops, leading to substantial changes in the operation of the NFAT circuit. If this were the case, it would also explain the need to express *DSCR1* and *DYRK1A* at somewhat more than a 1.5-fold excess to give rise to heart defects in our studies. Mathematical simulations of the operation of the NFAT genetic regulatory circuit based on our current knowledge indicate that it is robust and will function well under the wide variety of initial concentrations that might be encountered in the development of different tissues and organs. However, it is particularly susceptible to an increase in the activity of the two synergistically functioning regulators of NFATc nuclear occupancy, DSCR1 and DYRK1A, the genes for which happen to lie close to one another on HSA21. Our observations suggest that other human diseases may arise from the specific susceptibilities of genetic regulatory circuits, and that molecular understanding of these circuits will help to predict their weaknesses as well as possible sites of therapeutic intervention.

METHODS

Nfatc1-, *Nfatc2*-, *Nfatc3*- and *Nfatc4*-knockout mice have been described[16,20,21]. Behavioural testing was performed as described[42]. E15.5 mouse cortical neurons were cultured and transfected as previously described[16]. Nuclear export assays of NFATc4 were performed as previously described[11]. Rabbit polyclonal antibodies against DYRK1A were generated against recombinant rat DYRK1A. *In vitro* kinase assays were performed as previously described[10]. Transgenic embryos were generated by microinjection of full-length rat *Dyrk1a* and/or murine *Dscr1* cDNA under control of a β-actin promoter into fertilized oocytes.

The mathematical model was constructed by means of a coupled set of five first-order, nonlinear ordinary differential equations with gain and loss terms that were solved numerically with the standard fourth-order Runge–Kutta method, implemented with a C++ code of about 500 lines run on a Linux workstation.

Detailed methods can be found in the Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature. A summary figure is also included.

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Author Contributions The order of listing of the authors J.R.A., M.M.W., A.P. and I.A.G. does in no way reflect their relative contribution to this work. I.A.G. and G.R.C. are responsible for the original concept. I.A.G. generated the *Nfatc* mutant mice (Fig. 1, Supplementary Figs 2–5 and Table 1), J.R.N. the *Cnb1* mutant mice (Supplementary Fig. 8c), and H.W. and L.C. the *Dyrk1a/Dscr1* transgenic mice (Fig. 3). I.A.G., M.M.W., C.-P.C., X.G., J.R.N., J.J.H., S.K.K., N.Y. and T.M. analysed mutant mice (Figs 1, 3 and Supplementary Figs 2–5 and Table 1). M.M.W. performed the skull morphometry studies (Fig. 1a–c and Supplementary Figs 2–4) and helped with the analysis of Ts1Cje and Ts65Dn mice (Supplementary Fig. 9). I.A.G. performed the neuron signalling experiments (Fig. 2a, b, e, f and Supplementary Figs 7, 8b), biochemical analysis of human Down's syndrome samples (Fig. 4c), calcineurin mutant mice (Supplementary Fig. 8c), Ts1Cje and Ts65Dn mice (Supplementary Fig. 9) and *Dyrk1a/Dscr1*-overexpressing mice (Fig. 3a) as well as the *Nfatc4* promoter studies (Supplementary Fig. 8). A.P. generated and solved the mathematical model (Fig. 4a, b, d and Supplementary Discussion B). U.F. provided the clinical samples (Fig. 4c). J.R.A. conducted the *in vitro* kinase (Fig. 2c, d and Supplementary Fig. 7) and 293T (Supplementary Fig. 6) assays and DSCR1/DYRK1A quantifications (used in Supplementary Discussion B), made the anti-DYRK1A antiserum and helped H.W. and I.A.G. to genotype some of the *Dyrk1a/Dscr1*-overexpressing mice. G.R.C., I.A.G., J.A.A., M.M.W. and A.P. wrote the manuscript and I.A.G., M.M.W., A.P. and G.R.C. generated the figures.

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