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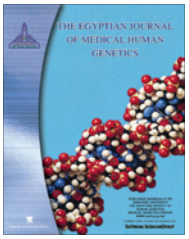

REVIEW

Models to explore the molecular function and regulation of AIRE

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Abstract Mutations in the *Autoimmune Regulator* (*AIRE*) gene are responsible for Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED). Within the thymic medulla, AIRE regulates the expression of a large number of tissue-specific self-antigens (TSAs) and the recognisation of these TSAs by auto-reactive T-cells is a prerequisite step for thymic negative selection. APECED patients will therefore develop multi-organ autoimmune disease because of the defective role of AIRE in thymic negative selection. Aire-deficient mice also develop multi-organ autoimmune disease and in this review we will focus on how both animal and cellular models have been used to dissect biochemical function of AIRE/Aire which is an essential step toward the understanding disease pathogenesis.

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1. Introduction

Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED) (also known as Autoimmune Polyglandular syndrome 1 – APS1) is an autosomal syndrome, diagnosed by the occurrence of two components of the clinical triad of chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency (Addison's disease) [1–3], and was first reported in 1946 [4]. A wide variety of secondary disorders have been identified in APECED patients, but as these manifest with lower incidences, they are not required for diagnosis of APECED. These other clinical diseases include a variety of autoimmune endocrinopathies, ectodermal dystrophies and gastrointestinal diseases (Table 1), as well as Sjögren's syndrome and keratoconjunctivitis. APECED patients also have a high incidence of squamous-cell carcinoma of the oral mucosa [1,2,5–10].

Studies reporting in 1997 had identified mutations in the *Autoimmune Regulator* (*AIRE*) gene as the causative factors of APECED [11,12]. Since then, a wide variety of different models systems have been used to investigate both human AIRE and murine Aire, varying from *Aire* knockout mice, developed as a systemic model to mimic APECED, to cell lines expressing recombinant AIRE or Aire and derivative variants, to in vitro methods using purified proteins. Over the course of these studies, AIRE/Aire has been identified to play an important role in negative selection in the thymus and the development of central tolerance. Model systems to study AIRE/Aire are therefore important, not only in the context of APECED, but also as a single causative gene disease model that can be used to discern key regulatory aspects of the central tolerance mechanism. Here, we review the models systems used to study the regulation and biochemical function of AIRE and Aire, what we have learned from these, and discuss the relevant merits and limitations of each system.

1.1. Tissue and cellular distribution of AIRE/Aire

The knowledge of the tissues and cells displaying AIRE/Aire expression (summarised in Table 2) can be particularly useful when making decisions upon which model system to use for further functional studies into AIRE/Aire. If using mice, these decisions can include which tissues to investigate in the study, or if using cell lines, whether to choose a cell line derived from a tissue that expressed AIRE/Aire natively, or to use a cell line identified to have some degree of AIRE/Aire expression.

Thymic expression of AIRE/Aire at both the RNA and protein level has been confirmed in both human and mice and identified in several other tissues across many studies investigating AIRE/Aire expression. However, it must be noted that the majority of studies investigating AIRE/Aire expression used mice, predominately the C57BL/6 strain, although Aire expression in the BALB/c, CD1, NIMR (Naval Medical Research Institute), NOD (Non-Obese Diabetic) and TRAMP (TRansgenic Adenocarcinoma of the Mouse Prostate) mice has been explored. Additionally, approximately half of the studies investigating extra-thymic AIRE expression in humans only examined PBMCs (Peripheral Blood Mononuclear Cells).

The reported extra-thymic expression of AIRE/Aire has proven to be much more contentious than that of thymic expression, as there has been an element of disparity between the different studies, with a recent review discussing these extra-thymic AIRE/Aire expression studies in more depth [13]. A major contributory factor to these differences between reports is the sensitivity of the assay used to detect AIRE/Aire expression. This is most clearly seen when certain studies using whole tissue homogenates could not detect AIRE/Aire in certain tissues, yet techniques that were able to detect AIRE/Aire at the cellular level, identified AIRE/Aire expression in specific cell sub-types of these tissues, typically either epithelial cells or cells of the myeloid lineage. Additionally, AIRE/Aire expres-

Table 1 Diseases associated with APECED that are not part of the diagnostic triad [1,2,5–10].

Type of disease	Disease
Autoimmune endocrinopathies	IDDM, thyroid diseases, pituitary defects and hypergonadotrophic hypogonadism
Ectodermal dystrophies	Alopecia areata, vitiligo, keratopathy, nail and dental enamel dystrophy
Gastro-intestinal diseases	Pernicious anaemia, malabsorption, cholelithiasis, hepatitis and chronic atrophic gastritis

Table 2 Tissues and specific cell types testing positive for AIRE/Aire expression in a report.

Organ/tissue	Cell types/regions showing Aire expression	
	RNA detected [14,36,41,65–82]	Protein detected [14,28,30,40,41,64,67,68,71,73,75,77,80,81,83,84]
Thymus	Detected in tissue. Also reported in medulla and as a scattered distribution amongst epithelial cells of the medulla (MECs); Dendritic cells; high levels found in $CD80^{int}$ and $CD80^{hi}$ MECs	Detected in tissue. Also reported in medulla and MECs; occasional medullary and cortical non-epithelial cells; (margins of) Hassal's corpuscles. Found in $CD80^{hi}MHC\ II^{hi}$ cells; $ly51^{low}MHCII^{hi}$ cells; $Cld3,4^{+}$ cells; $CD45^{-}$ cells; $CD4^{+}CD8^{+}$ thymocytes
Spleen	Detected in tissue. Also reported in border of red and white pulp; $CD11c^{+}$ cells; Dendritic cells	Detected in T-cell areas, T-cells and B-cells of white pulp; lymphocytes, some neutrophilic granulocytes and macrophages of red pulp; MHC II^{+} cells
Lymph nodes	Detected in tissue. Also reported in medulla and paracortical region; $CD45^{+}$ cells; $gp38^{-}CD31^{-}$ cell; very low levels found in $gp38^{+}CD31^{-}$ fibroblastic reticular cells, $gp38^{-}CD31^{+}$ blood endothelial cells and $gp38^{+}CD31^{+}$ lymphatic endothelial cells	Detected in medulla; also lymphocytes and some reticular cells of medulla; T-cell areas; light zone and some of dark zone of germinal centres; MHC II^{+} cells[a]
Kidney	Detected in tissue	Detected in epithelial cells of tubules; podocytes of glomerulus; transitional epithelium of bladder
Lung	Detected in tissue	Epithelial cells of large airways; alveolar macrophages
Liver	Detected in adult tissue and in foetal liver	Hepatocytes; Küppfer cells
Ovary, fallopian tubes and uterus	Detected in ovary and granulosa cells	Detected in ovary, also in epithelial cells of fallopian tube; follicular cells; oocytes Uterus – epithelial cells of mucosa and secretory glands; myometrium smooth muscle
Testis and prostate	Detected in testes	Detected in testes, also reported in spermatocytes and spermatids in seminiferous tubules; spermatogonia; Sertoli cells. Epithelial cells of protaste, epididymis and seminal vesicle
Brain/nervous system	Detected in tissue	Cerebral cortex; hippocampus; amygdale; thalamic nuclei; spinal cord; dorsal root ganglia; cortex, purkinje and granular neurones
Pituitary		Anterior and intermediate lobes
Thyroid	Detected at low levels in tissue	Parafollicular cells; epithelial cells of follicles
Adrenal gland	Detected (at low levels) in tissue	Zona glomerulosa; medullar chromaffin cells
Pancreas		Exocrine and endocrine cells
Bone marrow	Detected in tissue	Some large megacaryocytes; lymphoblasts; and myeloblasts
Skeletal muscle	Detected in tissue	
Peripheral blood	CD14+ Monocytes; Dendritic cells (including monocyte derivied, plasmacytoid and lymphoid); CD4+ T-cells (both $CD25^{+}$ and $CD25^{-}$); and (weakly) in B-cells	$CD11c^{+}$ (Dendritic) cells; lymphocytes; monocytes; polymorphonuclear leukocytes; $CD4^{-}CD8^{-}$ cells (B-cells); and granulocytes
Heart	Detected (at low levels) in tissue	
Gut/Alimentary tract	Detected (at low levels) in tissue. Also found in gut associated lymphoid tissues (GALT)	Detected in secretory glands (accini and secretory duct secretory cells); Alimentary canal (Epithelial and glandular cells); goblet cells; GALT of large & small bowel and appendix
Appendix	Low levels in homogenate	
Skin and hair		Bulb of hair follicles and outer root sheath
Cell lines	Jurkat; ATN-1; OTC-4; TW-EBV-LCL; MDK3; THP-1; HaCaT	MDK3; IL-2T; THP-1; HaCaT

[a] See Poliani et al. [68] for a comprehensive list of cell surface markers found on $AIRE^{+}$ cells in lymph node.

sion findings at the protein level did not always tally with those at the RNA level, with inadequate anti-AIRE/Aire antibodies suggested as a likely reason for this [13].

This knowledge of the tissues and cells displaying AIRE/Aire expression (summarised in table 2) can be used to decide upon which model system to use for further functional studies

into AIRE/Aire, with certain cell lines also observed to express AIRE/Aire to some degree.

1.2. Subcellular localisation of AIRE/Aire

Whilst the subcellular distribution of AIRE from immunohistochemical analysis of tissue sections has also been reported [14], the majority of studies investigating its subcellular localisation have used transfected cell-lines expressing recombinant variants of AIRE/Aire. These have included wild type AIRE/Aire, variants containing reported APECED mutations, and those with specific regions deleted in order to discern the function of specific domains in subcellular localisation [14–22].

Despite recombinant copies of both human AIRE and murine Aire being artificially expressed in differing species and tissue origins, consistent patterns of subcellular localisation was observed. AIRE was predominantly found in the nucleus where it was often described as a "speckled pattern" forming distinct nuclear bodies/dots (NBs), although it can also be found in the cytoplasm, where it has a more filamentous distribution [14,16,18,20,21]. Cytoplasmic AIRE was found on fibres that stretched from the nuclear envelope to the plasma membrane and co-localised with microtubules and with vimentin, a component of intermediate filaments [14,18,20,21]. A GFP-*AIRE* expression vector transfected into HeLa cells identified that whilst AIRE did form NBs, this was dependent on cell cycle and NBs would form after cell division but disappear before mitosis [16].

2. Studies using mouse models

2.1. Pathways affecting Aire expression

Mouse models have been used extensively in studies involving Aire, with a variety of transgenic mice used to great effect to trace the development of Aire expressing cells, particularly of those in the thymus. $Lta^{-/-}$ and $Ltbr^{-/-}$ mice were found to have a reduction in thymic Aire expression, suggesting the lymphotoxin pathway regulated Aire expression [23]. It was subsequently determined that the lymphotoxin pathway appears to be involved in the organisation and/or development of the thymus, and that Aire expressing cells differentiate from a sub-set of the cells regulated by this pathway. In a related study, NFκB2 (Nuclear Factor κ B2) was found to be an important part of this development pathway, through the use of $NF\kappa B2^{-/-}$ mice [24].

Studies involving $RANK^{-/-}$ (Receptor Activator of Nuclear Factor κ B) and $TRAF6^{-/-}$ (TNF Receptor Associated Factor 6) mice identified that RANK/RANKL (RANK Ligand) regulates Aire expression amongst mTEC precursor cells during development via TRAF6, and that RANK signalling is important for maintenance and turnover of $Aire^{+}$ mTECs in adults [25–27]. It was also discerned using transgenic mice that $Aire^{+}$ thymic cells were typically derived from Claudin-3^{+}, Claudin-4^{+} (Cld3,4^{+}) cells, with the minority of Cld3,4^{-} $Aire^{+}$ cells being of dendritic cell origin [28].

2.2. Aire knockout mice

Within a relatively short period after the identification of AIRE as the causative gene in APECED, *Aire* knockout mouse strains were developed as model organisms for the study of APECED. These have subsequently been used in studies investigating the molecular function of Aire and the pathways it is involved in.

The original *Aire* knockout mice were generated using the C57BL/6 mouse strain, and observed to produce auto-antibodies directed towards a variety of organs, as well as displaying lymphocytic infiltration in a number of organs too [29,30]. Comparisons between *Aire* knockout mice generated on different genetic backgrounds (C57BL/6, BALB/c, NOD) identified that there was variation in the targets of autoimmune attack between the different strains, indicating that the genetic background can modulate autoimmune symptoms [31]. These knockout mice also identified that Aire regulated the promiscuous expression of tissue specific antigens (TSAs) in the thymus, and that this function has a role in the negative selection of thymocytes in the thymus. Whilst Aire typically up-regulated gene expression, up to one third of the genes found to be differentially expressed were down-regulated in $Aire^{+/+}$ mice compared to the $Aire^{-/-}$ mice [32–34].

The gene expression profile of *Aire* knockout mice compared against their reciprocal wild type mice have since been used in a number of studies to elucidate the repertoire of Aire regulated genes [29,32–39]. Studies investigating the targets of Aire regulated gene expression in medullary thymic epithelial cells (mTECs) identified that the differentially expressed genes tended to co-localise into chromosomal clusters (of approx. 200kb in size), but did not indicate a particular chromosomal preference for Aire [32]. The level of expression of Aire regulated genes has been shown to vary between not only between different mice of the same genetic strain, but has a similar degree of variation between the mTECs taken from the two thymic lobes of the same mouse. Nevertheless, comparisons made between the repertoires of Aire regulated genes in the mTECs generated from mice of different genetic backgrounds (C57BL/6, BALB/c and NOD) [34], showed elements of conservation as well as divergence between the repertoires from these different genetic backgrounds. Aire regulated gene expression has also been compared between the mTECs and pancreatic islet beta-cells of mice on the NOD genetic background, finding quite a large divergence between the repertoires of differentially expressed genes, although approx 1/3 of these genes were down regulated as would be expected. Despite alternate methods used to determine these repertoires, wild type vs $Aire^{-/-}$ for the mTECs and Rat Insulin Promoter driven expression of recombinant Aire (RIP-Aire) vs wild type for pancreatic beta cells, the level of divergence between these two repertoires suggests that the repertoire of Aire regulated genes is strongly influenced by tissue type [33].

The results from a number of studies using a range of transgenic mice that are either wild type ($Aire^{+/+}$), heterozygote knockout ($Aire^{+/-}$) or homozygote knockout ($Aire^{-/-}$), have suggested that Aire functions in a dose dependent fashion. Specific peripheral tissue antigens (Tff3, Ins2, Mup1, Spt1) were found to be expressed at lower levels in $Aire^{+/-}$ mice than in wild type mice, and at even lower levels in $Aire^{-/-}$ mice [40,41].

3. Studies using cell line models

Cell line models have mainly been used in two different ways in the study of AIRE/Aire function. The first is as a tool to ex-

Table 3 Summary of studies looking at specific proteins that interact with AIRE/Aire.

Protein	Function	Cell line used	Findings
CBP (CREB Binding Protein)	Transcription co-factor	HEK293	AIRE binds to CBP via CH1 and CH3 domain on CBP [42]. AIRE and CBP act synergistically to active *IFNβ* (interferon β) expression [35], and affects expression of other genes [59]
DAXX	Role in repression of transcription	Cos-1 and HeLa	N-terminal region of AIRE interacts with SPT domain of DAXX in Cos-1 cells. DAXX suppressed AIRE mediated activation of *Insulin* promoter, and suggestion that this is via histone deacetylation [53]
DNA-PK complex(DNA Dependant Protein Kinase)	Protein kinase	THP-1 and HEK293	AIRE PHD1 domain interacted with Ku70, Ku80 and DNA-PKcs in THP-1 cells. In HEK293 cells, full length AIRE interacts with DNA-PKcs, and AIRE is phosphorylated by the DNA-PK complex in the N-terminus at residues T68 and S156 [46]
p63	Transcription factor	HEK293 and P1.4 (human thymic epithelial cells.)	SAND domain of AIRE interacts with TA domain of p63, and is prevented by G228W-AIRE mutation. HLA class II expression is reduced by p63 alone, but increased with co-expression of p63 and AIRE [85]
PIAS-1(Protein Inhibitor of Activated STAT)	E3 SUMO ligase	Cos-1 and Caco2 (human colon epithelial cells)	CARD domain of AIRE interacts with c-terminal domain of PIAS-1. AIRE and PIAS-1 activate expression from Insulin promoter concurrently, whereas AIRE represses *CSTB* (Cystatin B) promoter, which PIAS-1 activates. AIRE does not undergo sumoylation [46]
pTEF-b(Positive Transcription Elongation Factor b)	Prepares RNAPII for transcription elongation	HeLa-MAGI and 1C6 (mouse mTECs)	AIRE promotes transcription elongation. AIRE interacts with cyclin T1 and cyclin dependent kinase 9 components of P-TEFb complex, and recruits this complex to mouse *Ins2* (Insulin 2) and *Spt1* (salivary protein 1) promoters [54]

press recombinant proteins and study the molecular interactions of these specific recombinant proteins, typically in the identification of proteins that AIRE/Aire is able to bind to, and the critical domains for this interaction. The second is as a cellular model with which to discern either the factors that affect AIRE/Aire expression, or the cellular effects of AIRE/Aire.

3.1. Interaction of AIRE/Aire with other macro-molecules

One of the earliest complexes identified to be formed by AIRE/Aire, was that of a homodimer and homotetramer, with this ability first determined using in vitro translated protein, and verified using hybrid assays in yeast and mammalian cells (HUH-7 and Cos-1) [42]. This oligomerisation was further corroborated in later studies using recombinant protein expressed in *E. coli* [43], and mammalian 2-hybrid assays using COS-1 cells [44].

In addition to its homo-multimerisation, AIRE has been found not only to associate with the nuclear matrix during protein extractions [16,45,46], but also bind to DNA [43,47] and Histone H3 [48–52]. Studies involving in vitro assays using *E. coli* expressed recombinant protein, identified that whole AIRE as well as certain domain deletion variants, bound to DNA in a sequence specific manner via the SAND domain and two PHD zinc finger domains [43,47].

In vitro assays were used to identify that the PHD-1 domain of AIRE bound to Histone H3, that this binding is inhibited by methylation of the lysine4 residue of H3 (H3K4), and also in the determination of the structure and interaction of AIRE-PHD1 with a H3K4me0 peptide by NMR [49–51]. However, these findings lead to the exploration of recombinant AIRE in HEK293 cells, finding that AIRE was typically localised in areas with low H3K4 methylation, and in ChIP assays, that AIRE interacted with the promoters of the up-regulated genes *Insulin, Involucrin* and *S100A8*, which "almost completely lacked H3K4me3", but showed less interaction, and an enrichment of H3K4me3, for the promoters of the unaffected genes *S100A10* and *GAPDH* [49]. Co-expression of PRMT6 (H3R2 methyltransferase) with AIRE in HEK293 cells was found to reduce AIRE mediated up-regulation of Involucrin and S100A8. In addition, co-expression of the histone demethylase, PLU-1, with Aire in the U2OS (a human osteosarcoma) cell line, was found to increase the repertoire of Aire regulated genes than with just Aire alone [48].

The studies exploring the interaction of AIRE/Aire with other proteins, often used screening with yeast 2-hybrids [46,53] or pull-down assays/co-immunoprecipitation [52,54,55] to initially identify proteins that interacted with AIRE/Aire (Table 3). However, Pitkänen et al. selected CBP (CREB Binding Protein) for a series of in vitro assays, as a potential co-factor that interacted with AIRE as it was "the most common coactivator" [42]. After a target protein/complex had been identified, cell lines were typically then used either to corroborate in vitro findings, or help discern particular protein domains involved in the interaction, or to identify cellular effects of this interaction. A broad screen of proteins co-immunoprecipitating with recombinant Aire-FLAG fusion protein in HEK293 and 1C6 cells identified a range of proteins that interact with Aire, and tested the effect of these identified proteins on recombinant Aire mediated expression of *KRT14* (Keratin 14) in HEK293 cells by knocking down their expression with shRNAs. The identified proteins were grouped into four broad functional classes of chromatin binding/structure, nuclear transport, pre-mRNA processing and transcription, and Aire's involvement in these biochemical processes further tested. Aire was found to elevate

the levels of spliced (mature) mRNA, but not unspliced (pre-) mRNA in HEK293 cells, and in the human 4D6 (thymic epithelial) cell line, knockdown of the NPC-associated proteins NUP93, XPO1, KNPB1 and RanBP2, caused cytosolic retention of Aire, suggesting an indirect effect on Aire mediated regulation of expression [52].

3.2. Regulation of AIRE/Aire expression

A number of studies have used cell lines to either identify mechanisms that induce AIRE expression, or factors that are involved in the regulation of AIRE expression.

Investigations by the Pärt Peterson research group have used COS-7 cells, HeLa cells, the THP-1 and U937 human monocytoid cell lines and the TEC 1A3 human thymic cell line to study the regulation of AIRE transcription. They determined the minimal AIRE promoter, consisting of the first 350bp upstream of the ATG start codon (223bp upstream of the transcription start site). Additionally, they identified and validated transcription factor binding sites in this region for Ets-1, AP-1, Sp-1 (GC box), NF-y (an inverted CCAAT box) and a TATA box motif [56,57]. They also discerned that regulation of AIRE expression is mediated by methylation of the AIRE promoter, and were able to up-regulate AIRE expression by treating cells with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine, which was further increased upon addition of trichostatin A, a histone deactylase inhibitor, to the treatment regimen [56]. Nagafuchi et al. [58] were able to induce AIRE expression in the OTC-4 myelomonocytic leukaemia cell line for a limited time (less than 72 h from the commencement of treatment) by treating the cells with granulo-monocyte colony stimulating factor (GM-CSF), triggering the cell line to differentiate from monoblast-like cells into monocytes. They were able to determine that this expression was mediated through the p38 MAPK pathway, through the identification of phosphorylated proteins in the treated cells, and co-treatment with specific inhibitors alongside GM-CSF.

3.3. AIRE/Aire regulated gene expression

One of the earliest studies to identify targets of AIRE/Aire regulated gene expression in cell lines used ribonuclease protection assays to determine differential expression of a limited selection of cytokine and chemokine genes in the murine Raw 264.7 macrophage cell line [59]. Comparisons were made between the unmodified cell line and a variant carrying a stable transfectant of a recombinant Aire expression vector. IL-1ra, whose expression is normally regulated in a CBP dependent manner, was one of the few genes indentified to have a significant change in expression as a result of recombinant Aire expression. It was hypothesised that Aire dependent down-regulation of expression of this, and other genes, might be mediated by Aire sequestering CBP and quenching its normal function.

A report into AIRE regulated gene expression in the U937 cell line was released shortly after this study was published, and used a custom cDNA chip to investigate the effect of stable expression of recombinant AIRE on the cells transcriptome [60]. Similar to findings in mice, AIRE was found to both up- and down-regulate gene expression, with approx 1/3 of the genes affected being down-regulated (48 genes up-regulated and 24 down regulated). Later studies using HEK293 cells found that recombinant AIRE up-regulated the expression of *Insulin*, *Involucrin* and *S100A8* by QPCR [49], and using Illumina beadchip arrays, identified significant up- and down-regulation of 140 and 154 genes respectively [61]. The genes identified in the beadchip array were also found to be more clustered than would be expected at random, and that AIRE down-regulated genes often had high levels of initial expression, and up-regulated genes typically had low levels of initial expression and lacked H3K4me3 and AcH3 markers on histones near to their promoters. Recombinant Aire expression in HEK293 cells was found to generate a 2-fold or greater up-regulation of expression in ~900 genes and similar down-regulation of expression for ~300 genes [52].

4. Choice of model system

There are many good reasons why mice have been used so extensively out of all the studies investigating AIRE/Aire function. Firstly, they provide a reproducible and modifiable model system that can have cells which have prolonged native high level expression of Aire. Secondly, they are the only genetically manipulatable system which can be used in investigations into the systemic effects of AIRE/Aire and its role in the development of central tolerance and autoimmune pathogenesis. Additionally, the variety of different genetic strains of mice enabled the identification that genetic background had a significant effect on Aire function. However, this can be both a curse and a benefit. On one hand this could complicate the interpretation of results, for example, whether certain downstream effects of Aire have been modulated due to the genetic background. Whereas on the other hand, experiments using a wide variety of different mouse strains could help identify the full range of the downstream effects of Aire, and comparisons of related experiments using different strains of mice can identify conserved Aire functionality.

A particular disadvantage using mice to determine the biochemical targets and downstream pathways of Aire is that there is likely to be increased heterogeneity between the cells extracted and tested. Aire has been found to be expressed in only specific cells of certain tissues, and thus there is the potential for non-relevant cells to modulate observed results. In addition, intra-tissue variation has already been reported, and thus this increased background noise may serve to reduce the sensitivity to detect all Aire dependent effects. Finally, whilst one must always be conscious of the potential for variation in a gene's effects when using mice (or another organism) and their corresponding gene homolog as a model for the study of a human disorder and/or gene, particular consideration must be taken for the study of AIRE and APECED. There has been published debate over the relevance of Aire knockout mice as an appropriate model for APECED [62–64], particularly as these mice have not displayed the full range of symptoms of APECED. However, as pointed out, genetic background can modify disease symptoms observed in these knockout mice, thus the lack of a wide range of genetically diverse *Aire* knockout mice, compared to the genetic diversity amongst APECED patients, may in part explain this absence of a full range of symptoms.

Cell line models can overcome some of the problems that could affect mouse models, although they too have certain drawbacks in their use as model systems. One of the major benefits of using cell lines, is that it is possible to test the bio-

chemical interactions of human AIRE within a human cell line, or those of murine Aire without the need of whole mice. This is coupled with the fact that a single mouse will have a limited number of $Aire^+$ cells from a particular tissue, whereas cell culture can generate many more $AIRE^+$ or $Aire^+$ cells, and thus provide sufficient cell numbers from a single source for use in experiments, as has previously reported [52]. Additionally it may be possible to obtain cell lines that have been derived from appropriate tissues, such as the thymus, which as human tissue samples could be harder to resource. Unfortunately, there is still a limitation to the range of the different sub-populations that make up a tissue that are available as a cell line. Thus, as AIRE/Aire is only expressed in certain cell types within a specific tissue, there may need to be compromise in the design of experiments, as to how similar the cell line of a tissue is to the native cellular environment in the tissue where AIRE/Aire is natively expressed. This is obviously less of an issue if the study is focusing on the interaction between recombinant AIRE/Aire or modified variant with another recombinant protein, rather than AIRE/Aire's interaction with macro-molecules native to the cellular.

In addition to this, due to different cell lines being derived from different donor sources, when comparing results from two different cell lines, there is likely to be an element of genetic heterogeneity. These genetic differences could provide additional ''background noise'' that might modulate the observed results, and thus may affect the sensitivity to determine effects of the other differences between the cell lines, such as species or tissue type the cell line was derived from.

One major advantage to cell lines is that they are relatively simple to modify, whether to express recombinant proteins, or to knockdown gene expression by RNAi, or to perform different treatment regimens with various additives to growth media. Unfortunately, this is coupled with the drawback of the relative dearth of cell lines that display native AIRE or Aire expression. Whilst, AIRE expression has been induced in several cell lines after treatment [56,58], the resultant cell lines either expressed AIRE for only a relatively short period of time [58], or the treatments to demethylate DNA and deacetylate histones would have altered the cellular biochemistry to make it an inadequate model for AIRE function [56]. Consequently, many of the cell lines used to examine AIRE or Aire, needed to introduce an artificial system to express recombinant AIRE/Aire or another construct, such as a fusion protein, or protein variants containing mutations or deletions or specific domains, although there may be side-effects as a result of this. For example, artificial expression of recombinant AIRE/Aire, if driven by the CMV promoter (which is commonly used in expression vectors) will generate higher levels of expression than native AIRE/Aire expression, and thus the observed functional effects may differ than what would be observed under more native expression levels. However, this will be tempered if different experimental variants are required, whether using different variants of AIRE/Aire, or the expression of additional recombinant proteins.

In short, all studies need to carefully decide whether the cellular environment, whether murine or human tissue sample, or tissue derivation of cell line, is appropriate for the focus of the investigation. Mice can provide a model where the effects of natively expressed Aire can be easily investigated, and can identify Aire's systemic effects on other cells and tissues, in addition to the cellular environment. Whereas cell lines can be used to model AIRE as well as Aire and provide larger numbers of a more homogenous sample of cells in a system that can be more easily modified, although the choice of cell lines natively expressing AIRE or Aire is limited.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

[1] Scott HS, Heino M, Peterson P, Mittaz L, Lalioti MD, Betterle C, et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Molecular Endocrinology Baltimore, Md. 1998;12(8):1112–9.

[2] Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990;322(26):1829–36.

[3] Aaltonen J, Komulainen J, Vikman A, Palotie A, Wadelius C, Perheentupa J, et al. Autoimmune polyglandular disease type I. Exclusion map using amplifiable multiallelic markers in a microtiter well format. Eur J Hum Genet 1993;1(2):164–71.

[4] Leonard M. Chronic idiopathic hypoparathyroidism with superimposed Addison's disease in a child. J Clin Endocrinol Metab 1946;6:493–5.

[5] Zlotogora J, Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jews. J Med Genet 1992;29(11):824–6.

[6] Wang CY, Davoodi-Semiromi A, Huang W, Connor E, Shi JD, She JX. Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS1). Human genetics 1998;103(6):681–5.

[7] Myhre AG, Halonen M, Eskelin P, Ekwall O, Hedstrand H, Rorsman F, et al. Autoimmune polyendocrine syndrome type 1 (APS I) in Norway. Clin Endocrinol 2001;54(2):211–7.

[8] Neufeld M, Maclaren NK, Blizzard RM. Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. Medicine (Baltimore) 1981;60(5):355–62.

[9] Neufeld M, Maclaren N, Blizzard R. Autoimmune polyglandular syndromes. Pediatr Ann 1980;9(4):154–62.

[10] Gass JD. The syndrome of keratoconjunctivitis, superficial moniliasis, idiopathic hypoparathyroidism and Addison's disease. Am J Ophthalmol 1962;54:660–74.

[11] An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 1997;17(4):399–403.

[12] Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, et al. Positional cloning of the APECED gene. Nat Genet 1997;17(4):393–8.

[13] Eldershaw SA, Sansom DM, Narendran P. Expression and function of the autoimmune regulator (Aire) gene in non-thymic tissue. Clin Exp Immunol 2010;163(3):296–308.

[14] Heino M, Peterson P, Kudoh J, Nagamine K, Lagerstedt A, Ovod V, et al. Autoimmune regulator is expressed in the cells regulating immune tolerance in thymus medulla. Biochem Biophys Res Commun 1999;257(3):821–5.

[15] Ilmarinen T, Melén K, Kangas H, Julkunen I, Ulmanen I, Eskelin P. The monopartite nuclear localization signal of autoimmune

regulator mediates its nuclear import and interaction with multiple importin α molecules. FEBS J 2006;273(2):315–24.
[16] Akiyoshi H, Hatakeyama S, Pitkanen J, Mouri Y, Doucas V, Kudoh J, et al. Subcellular expression of autoimmune regulator is organized in a spatiotemporal manner. J Biol Chem 2004;279(32):33984–91.
[17] Ramsey C, Bukrinsky A, Peltonen L. Systematic mutagenesis of the functional domains of AIRE reveals their role in intracellular targeting. Human Mol Genet 2002;11(26):3299–308.
[18] Pitkanen J, Vahamurto P, Krohn K, Peterson P. Subcellular localization of the autoimmune regulator protein. Characterization of nuclear targeting and transcriptional activation domain. J Biol Chem 2001;276(22):19597–602.
[19] Björses P, Halonen M, Palvimo JJ, Kolmer M, Aaltonen J, Ellonen P, et al. Mutations in the AIRE Gene: Effects on Subcellular Location and Transactivation Function of the Autoimmune Polyendocrinopathy-Candidiasis–Ectodermal Dystrophy Protein. Am J Hum Genet 2000;66(2):378–92.
[20] Rinderle C, Christensen HM, Schweiger S, Lehrach H, Yaspo ML. AIRE encodes a nuclear protein co-localizing with cytoskeletal filaments: altered sub-cellular distribution of mutants lacking the PHD zinc fingers. Human Mol Genet 1999;8(2):277–90.
[21] Björses P, Pelto-Huikko M, Kaukonen J, Aaltonen J, Peltonen L, Ulmanen I. Localization of the APECED protein in distinct nuclear structures. Human Mol Genet 1999;8(2):259–66.
[22] Pitkänen J, Vähämurto P, Krohn K, Peterson P, Subcellular Localization of the Autoimmune Regulator Protein. Characterization of nuclear targeting and transcriptional activation domain. J Biol Chem 2001;276(22):19597–602.
[23] Chin RK, Lo JC, Kim O, Blink SE, Christiansen PA, Peterson P, et al. Lymphotoxin pathway directs thymic Aire expression. Nature Immunol 2003;4(11):1121–7.
[24] Zhu M, Chin RK, Christiansen PA, Lo JC, Liu X, Ware C, et al. NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. J Clin Invest 2006;116(11):2964–71.
[25] White AJ, Withers DR, Parnell SM, Scott HS, Finke D, Lane PJ, et al. Sequential phases in the development of Aire-expressing medullary thymic epithelial cells involve distinct cellular input. Eur J Immunol 2008;38(4):942–7.
[26] Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol 2001;2(11):1032–9.
[27] Akiyama T, Maeda S, Yamane S, Ogino K, Kasai M, Kajiura F, et al. Dependence of self-tolerance on TRAF6-directed development of thymic stroma. Science 2005;308(5719):248–51.
[28] Hamazaki Y, Fujita H, Kobayashi T, Choi Y, Scott HS, Matsumoto M, et al. Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. Nature Immunol 2007;8(3):304–11.
[29] Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al.s Projection of an immunological self shadow within the thymus by the aire protein. Science (New York, NY) 2002;298(5597):1395–401.
[30] Ramsey C, Winqvist O, Puhakka L, Halonen M, Moro A, Kampe O, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Human Mol Genet 2002;11(4):397–409.
[31] Jiang W, Anderson MS, Bronson R, Mathis D, Benoist C. Modifier loci condition autoimmunity provoked by Aire deficiency. J Exp Med 2005;202(6):805–15.
[32] Johnnidis JB, Venanzi ES, Taxman DJ, Ting JP, Benoist CO, Mathis DJ. Chromosomal clustering of genes controlled by the aire transcription factor. Proc Natl Acad Sci USA 2005;102(20):7233–8.
[33] Guerau-de-Arellano M, Mathis D, Benoist C. Transcriptional impact of Aire varies with cell type. Proc Natl Acad Sci USA 2008;105(37):14011–6.
[34] Venanzi ES, Melamed R, Mathis D, Benoist C. The variable immunological self: genetic variation and nongenetic noise in Aire-regulated transcription. Proc Natl Acad Sci USA 2008;105(41):15860–5.
[35] Pitkanen J, Rebane A, Rowell J, Murumagi A, Strobel P, Moll K, et al. Cooperative activation of transcription by autoimmune regulator AIRE and CBP. Biochem Biophys Res Commun 2005;333(3):944–53.
[36] Derbinski J, Gabler J, Brors B, Tierling S, Jonnakuty S, Hergenhahn M, et al. Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. J Exp Med 2005;202(1):33–45.
[37] Ruan QG, Tung K, Eisenman D, Setiady Y, Eckenrode S, Yi B, et al. The autoimmune regulator directly controls the expression of genes critical for thymic epithelial function. J Immunol 2007;178(11):7173–80.
[38] Gavanescu I, Kessler B, Ploegh H, Benoist C, Mathis D. Loss of Aire-dependent thymic expression of a peripheral tissue antigen renders it a target of autoimmunity. Proc Natl Acad Sci USA 2007;104(11):4583–7.
[39] Gillard GO, Dooley J, Erickson M, Peltonen L, Farr AG. Aire-dependent alterations in medullary thymic epithelium indicate a role for Aire in thymic epithelial differentiation. J Immunol 2007;178(5):3007–15.
[40] Liston A, Gray DH, Lesage S, Fletcher AL, Wilson J, Webster KE, et al. Gene dosage–limiting role of Aire in thymic expression, clonal deletion, and organ-specific autoimmunity. J Exp Med 2004;200(8):1015–26.
[41] Kont V, Laan M, Kisand K, Merits A, Scott HS, Peterson P. Modulation of Aire regulates the expression of tissue-restricted antigens. Mol Immunol 2008;45(1):25–33.
[42] Pitkanen J, Doucas V, Sternsdorf T, Nakajima T, Aratani S, Jensen K, et al. The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. J Biol Chem 2000;275(22):16802–9.
[43] Kumar PG, Laloraya M, Wang CY, Ruan QG, Davoodi-Semiromi A, Kao KJ, et al. The autoimmune regulator (AIRE) is a DNA-binding protein. J Biol Chem 2001;276(44):41357–64.
[44] Halonen M, Kangas H, Ruppell T, Ilmarinen T, Ollila J, Kolmer M, et al. APECED-causing mutations in AIRE reveal the functional domains of the protein. Human Mut 2004;23(3):245–57.
[45] Tao Y, Kupfer R, Stewart BJ, Williams-Skipp C, Crowell CK, Patel DD, et al. AIRE recruits multiple transcriptional components to specific genomic regions through tethering to nuclear matrix. Mol Immunol 2006;43(4):335–45.
[46] Ilmarinen T, Kangas H, Kytomaa T, Eskelin P, Saharinen J, Seeler JS, et al. Functional interaction of AIRE with PIAS1 in transcriptional regulation. Mol Immunol 2008;45(7):1847–62.
[47] Purohit S, Kumar PG, Laloraya M, She JX. Mapping DNA-binding domains of the autoimmune regulator protein. Biochem Biophys Res Commun 2005;327(3):939–44.
[48] Koh AS, Kingston RE, Benoist C, Mathis D. Global relevance of Aire binding to hypomethylated lysine-4 of histone-3. Proc Natl Acad Sci USA 2010;107(29):13016–21.
[49] Org T, Chignola F, Hetenyi C, Gaetani M, Rebane A, Liiv I, et al. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. EMBO Rep 2008;9(4):370–6.
[50] Chakravarty S, Zeng L, Zhou MM. Structure and site-specific recognition of histone H3 by the PHD finger of human autoimmune regulator. Structure 2009;17(5):670–9.
[51] Chignola F, Gaetani M, Rebane A, Org T, Mollica L, Zucchelli C, et al. The solution structure of the first PHD finger of autoimmune regulator in complex with non-modified histone H3 tail reveals the antagonistic role of H3R2 methylation. Nucleic Acids Res 2009;37(9):2951–61.

[52] Abramson J, Giraud M, Benoist C, Mathis D. Aire's partners in the molecular control of immunological tolerance. Cell 2010;140(1):123–35.
[53] Meloni A, Fiorillo E, Corda D, Incani F, Serra ML, Contini A, et al. DAXX is a new AIRE-interacting protein. J Biol Chem 2010;285(17):13012–21.
[54] Oven I, Brdickova N, Kohoutek J, Vaupotic T, Narat M, Peterlin BM. AIRE recruits P-TEFb for transcriptional elongation of target genes in medullary thymic epithelial cells. Mol Cellular Biol 2007;27(24):8815–23.
[55] Liiv I, Rebane A, Org T, Saare M, Maslovskaja J, Kisand K, et al. DNA-PK contributes to the phosphorylation of AIRE: importance in transcriptional activity. Biochim Biophys Acta 2008;1783(1):74–83.
[56] Murumagi A, Vahamurto P, Peterson P. Characterization of regulatory elements and methylation pattern of the autoimmune regulator (AIRE) promoter. J Biol Chem 2003;278(22):19784–90.
[57] Murumagi A, Silvennoinen O, Peterson P. Ets transcription factors regulate AIRE gene promoter. Biochem Biophys Res Commun 2006;348(2):768–74.
[58] Nagafuchi S, Katsuta H, Ohno Y, Inoue Y, Shimoda K, Kogawa K, et al. Mitogen-activated protein kinase pathway controls autoimmune regulator (AIRE) gene expression in granulo-monocyte colony stimulating factor (GM-CSF)-stimulated myelomonocytic leukemia OTC-4 cells. Immunol Lett 2005;99(1):130–5.
[59] Sato K, Sato U, Tateishi S, Kubo K, Horikawa R, Mimura T, et al. Aire downregulates multiple molecules that have contradicting immune-enhancing and immune-suppressive functions. Biochem Biophys Res Commun 2004;318(4):935–40.
[60] Sillanpaa N, Magureanu CG, Murumagi A, Reinikainen A, West A, Manninen A, et al. Autoimmune regulator induced changes in the gene expression profile of human monocyte-dendritic cell-lineage. Mol Immunol 2004;41(12):1185–98.
[61] Org T, Rebane A, Kisand K, Laan M, Haljasorg U, Andreson R, et al. AIRE activated tissue specific genes have histone modifications associated with inactive chromatin. Human Mol Genet 2009;18(24):4699–710.
[62] Pontynen N, Miettinen A, Arstila TP, Kampe O, Alimohammadi M, Vaarala O, et al. Aire deficient mice do not develop the same profile of tissue-specific autoantibodies as APECED patients. J Autoimmun 2006;27(2):96–104.
[63] Kekalainen E, Miettinen A, Arstila TP. Does the deficiency of Aire in mice really resemble human APECED? Nature Rev 2007;7(10):1.
[64] Kekalainen E, Tuovinen H, Joensuu J, Gylling M, Franssila R, Pontynen N, et al. A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Immunol 2007;178(2):1208–15.
[65] Cohen JN, Guidi CJ, Tewalt EF, Qiao H, Rouhani SJ, Ruddell A, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med 2010;207(4):681–8.
[66] Fletcher AL, Lukacs-Kornek V, Reynoso ED, Pinner SE, Bellemare-Pelletier A, Curry MS, et al. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. J Exp Med 2010;207(4):689–97.
[67] Kumar V, Pedroza LA, Mace EM, Seeholzer S, Cotsarelis G, Condino-Neto A, et al. The Autoimmune Regulator (AIRE), Which Is Defective in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Patients, Is Expressed in Human Epidermal and Follicular Keratinocytes and Associates With the Intermediate Filament Protein Cytokeratin 17. Am J Pathol 2010;178(3):983–8.
[68] Poliani PL, Kisand K, Marrella V, Ravanini M, Notarangelo LD, Villa A, et al. Human peripheral lymphoid tissues contain autoimmune regulator-expressing dendritic cells. Am J Pathol 2010;176(3):1104–12.
[69] Blechschmidt K, Schweiger M, Wertz K, Poulson R, Christensen HM, Rosenthal A, et al. The mouse Aire gene: comparative genomic sequencing, gene organization, and expression. Genome Res 1999;9(2):158–66.
[70] Ruan QG, Wang CY, Shi JD, She JX. Expression and alternative splicing of the mouse autoimmune regulator gene (Aire). J Autoimmun 1999;13(3):307–13.
[71] Heino M, Peterson P, Sillanpaa N, Guerin S, Wu L, Anderson G, et al. RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, RelB-deficient and in NOD mouse. Eur J Immunol 2000;30(7):1884–93.
[72] Zuklys S, Balciunaite G, Agarwal A, Fasler-Kan E, Palmer E. Hollander GA, Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). J Immunol 2000;165(4):1976–83.
[73] Halonen M, Pelto-Huikko M, Eskelin P, Peltonen L, Ulmanen I, Kolmer M. Subcellular location and expression pattern of autoimmune regulator (Aire), the mouse orthologue for human gene defective in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). J Histochem Cytochem 2001;49(2):197–208.
[74] Kogawa K, Nagafuchi S, Katsuta H, Kudoh J, Tamiya S, Sakai Y, et al. Expression of AIRE gene in peripheral monocyte/dendritic cell lineage. Immunol Lett 2002;80(3):195–8.
[75] Adamson KA, Pearce SH, Lamb JR, Seckl JR, Howie SE. A comparative study of mRNA and protein expression of the autoimmune regulator gene (Aire) in embryonic and adult murine tissues. J Pathol 2004;202(2):180–7.
[76] Zheng X, Yin L, Liu Y, Zheng P. Expression of tissue-specific autoantigens in the hematopoietic cells leads to activation-induced cell death of autoreactive T cells in the secondary lymphoid organs. Eur J Immunol 2004;34(11):3126–34.
[77] Nagafuchi S, Katsuta H, Koyanagi-Katsuta R, Yamasaki S, Inoue Y, Shimoda K, et al. Autoimmune regulator (AIRE) gene is expressed in human activated CD4+ T-cells and regulated by mitogen-activated protein kinase pathway. Microbiol Immunol 2006;50(12):979–87.
[78] Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, et al. RANK signals from CD4(+)3(−) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. J Exp Med 2007;204(6):1267–72.
[79] Pontynen N, Strengell M, Sillanpaa N, Saharinen J, Ulmanen I, Julkunen I, et al. Critical immunological pathways are downregulated in APECED patient dendritic cells. J Mol Med (Berlin, Germany) 2008;86(10):1139–52.
[80] Schaller CE, Wang CL, Beck-Engeser G, Goss L, Scott HS, Anderson MS, et al. Expression of Aire and the early wave of apoptosis in spermatogenesis. J Immunol 2008;180(3):1338–43.
[81] Suzuki E, Kobayashi Y, Kawano O, Endo K, Haneda H, Yukiue H, et al. Expression of AIRE in thymocytes and peripheral lymphocytes. Autoimmunity 2008;41(2):133–9.
[82] Campbell IK, Kinkel SA, Drake SF, van Nieuwenhuijze A, Hubert FX, Tarlinton DM, et al. Autoimmune regulator controls T cell help for pathogenetic autoantibody production in collagen-induced arthritis. Arthritis Rheumat 2009;60(6):1683–93.
[83] Gray D, Abramson J, Benoist C, Mathis D. Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. J Exp Med 2007;204(11):2521–8.
[84] Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X, et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science (New York, NY) 2008;321(5890):843–7.
[85] Tonooka A, Kubo T, Ichimiya S, Tamura Y, Ilmarinen T, Ulmanen I, et al. Wild-type AIRE cooperates with p63 in HLA class II expression of medullary thymic stromal cells. Biochem Biophys Res Commun 2009;379(3):765–70.