

TABLE 11.2 (Continued)

Functional Category	Gene	Description	FC
	<i>Vamp1</i>	Vesicle-associated membrane protein 1	0.5
	<i>Gfra1</i>	Glial cell line derived neurotrophic factor family receptor 1	0.45
	<i>Inha</i>	Inhibin alpha	0.45
	<i>Cd36</i>	cd36 antigen	0.4
	<i>Slc1a3</i>	Solute carrier family 1, member 3	0.34
Cell–cell signaling	<i>Spp1</i>	Secreted phosphoprotein 1	4.6
	<i>Gria3</i>	Glutamate receptor, ionotropic. AMPA3 (alpha 3)	2.9
	<i>Inha</i>	Inhibin alpha	0.45
	<i>Pdyn</i>	Prodynorphin	0.43
	<i>Slc 1a3</i>	Solute carrier famil 1, member 3	0.34
Development/morphogenesis	<i>Srd5a1</i>	Steroid 5 alpha-reductase 1	6.4
	<i>Spp1</i>	Secreted phosphoprotein 1	4.6
	<i>Mtap2</i>	Microtubule-associated protein 2	2.5
	<i>Cxcl10</i>	Chemokine (C-X-C motif) ligand 10	2.3
	<i>Il18</i>	Interleukin-18	2.0
	<i>Ghr</i>	Growth hormone receptor	2.0
	<i>Ania4</i>	Activity and neurotransmitter-induced early gene protein 4	2.0
	<i>Gfra1</i>	Glial cell line derived neurotrophic factor family receptor 1	0.45
	<i>Inha</i>	Inhibin alpha	0.45
	<i>Sfrp4</i>	Secreted frizzled-related protein 4	0.37
Structural/cell adhesion/extracellular matrix	<i>Spp1</i>	Secreted phosphoprotein 1	4.6
	<i>C1s</i>	Complement component 1, s subcomponent	3.0
	<i>Lox</i>	Lysyl oxidase	2.8
	<i>Cx3cl1</i>	Chemokine (C-X3-C motif) ligand 1	2.2
	<i>Il18</i>	Interleukin-18	2.0
	<i>Vamp1</i>	Vesicle-associated membrane protein 1	0.5
	<i>Cd36</i>	cd36 antigen	0.4
	<i>Bcan</i>	Brevican	0.23
	<i>Mmp9</i>	Matrix metalloproteinase 9	0.13
Lipid and cholesterol	<i>Pla2g1b</i>	Phospholipase A2, group IB	3.7
Metabolism/biosynthesis	<i>Gria3</i>	Glutamate receptor, ionotropic. AMPA3 (alpha 3)	2.9
	<i>Mch</i>	Medium-chain S-acyl fatty acid synthetase thio ester hydrolase	2.6

(Continued)

TABLE 11.2 (Continued)

Functional Category	Gene	Description	FC
	<i>Dig1</i>	Dithiolethione-inducible gene-1	2.3
	<i>Sc4mol</i>	Sterol-C4-methyl oxidase-like	0.5
	<i>Hmgcs1</i>	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	0.48
	<i>Cd36</i>	cd36 antigen	0.4
	<i>Idi1</i>	Isopentenyl-diphosphate delta isomerase	0.4
	<i>Cyp11b</i>	Cytochrome P450, subfamily XIB gene cluster	0.37
	<i>Pla2g4a</i>	Phospholipase A2, group IVA	0.37
	<i>Ptgs1</i>	Prostaglandin-endoperoxide synthase 1	0.36
	<i>Sqle</i>	Squalene epoxidase	0.36
Immune response	<i>Spp1</i>	Secreted phosphoprotein 1	4.6
	<i>Cxcl10</i>	Chemokine (C-X-C motif) ligand 10	2.3
	<i>Cx3cl10</i>	Chemokine (C-X3-C motif) ligand 1	2.2
	<i>RT1Aw2</i>	RT1 class Ib gene(Aw2)	2.2
	<i>Il18</i>	Interleukin-18	2.0
	<i>Inha</i>	Inhibin alpha	0.45

Abbreviation: FC, fold change.

Modified data published by Lee JJ, Widmaier EP. *J Steroid Biochem Mol Biol* 2005; 96: 31–44.

regulation of steroidogenesis. The genes down-regulated in response to ACTH contained significantly enriched clusters with functions in DNA replication, mitotic cell cycle, cell division, nuclear transport, and RNA processing, consistent with the growth-inhibiting effect of ACTH in Y1 cells.²¹ Schimmer *et al.* also determined the relative contributions of the cyclic adenosine monophosphate (cAMP) signaling pathway and the protein kinase C-dependent pathway to the ACTH effects as approximately 56% and 6%, respectively. Approximately 38% of the ACTH-affected transcripts could not be assigned to these signaling pathways and thus represent candidates for regulation via other mechanisms. The combined data from the two studies by Lee and Widmaier and Schimmer *et al.*, despite a certain level of inconsistency between these studies, provide useful reference

sets for more detailed studies of the factors that contribute to regulation of steroidogenesis and growth and development in the adrenal cortex upon ACTH stimulation.

TRANSCRIPTOME ANALYSIS OF DIFFERENT ZONES

Adrenocortical zonation remains an unresolved aspect for researchers investigating the mechanism underlying and the control element of zonation.²² Transcriptome analysis of different zones might help elucidate the molecular signature of functional zonation. The zona fasciculata and zona glomerulosa are present in rodents, whereas the zona reticularis is observed only in primates. Nishimoto *et al.* assessed the transcriptome of DEGs in the zona glomerulosa and zona

TABLE 11.3 Genes Regulated by ACTH in Y1 Adrenocortical Cell Line by cDNA Microarray

Gene	Description	FC	<i>p</i>	PKA	PKC
UPREGULATION					
<i>Adamts4</i>	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 4	8.68	0.0368	+	+
<i>Orc4l</i>	Origin recognition complex, subunit 4-like (<i>S. cerevisiae</i>)	5.55	0.0008	+	-
<i>Procr</i>	Protein C receptor, endothelial	4.96	0.0008	+	-
<i>Spp1</i>	Secreted phosphoprotein 1	3.98	0.0064	+	-
<i>Scarb1</i>	Scavenger receptor class B, member 1	3.85	0.0008	+	-
<i>Cdk8</i>	Cyclin-dependent kinase 8	3.68	0.0031	+	+
<i>Ier3</i>	Immediate early response 3	3.61	0.002	-	+
<i>Star</i>	Steroidogenic acute regulatory protein	2.92	0.0008	+	-
<i>ElaC2</i>	ElaC homolog 2 (<i>E. coli</i>)	2.91	0.0008	-	+
<i>Alas1</i>	Aminolevulinic acid synthase 1	2.62	0.0008	+	-
<i>Ucp2</i>	Uncoupling protein 2, mitochondrial	2.42	0.0015	+	-
<i>Cnnm2</i>	Cyclin M2	2.40	0.0111	-	+
<i>Ostf1</i>	Osteoclast stimulating factor 1	2.32	0.0008	+	+
<i>Prkar2b</i>	Protein kinase, cAMP dependent regulatory, type II beta	2.26	0.0008	+	+
<i>Eno1</i>	Enolase 1, alpha non-neuron	2.22	0.0368	-	-
<i>Hs1bp1</i>	HS1 binding protein	2.17	0.0008	+	+
<i>Gla</i>	Galactosidase, alpha	2.17	0.0008	+	-
<i>Sdc3</i>	Syndecan 3	2.11	0.0008	+	+
<i>Net1</i>	Neuroepithelial cell transforming gene 1	2.08	0.0008	+	-
<i>Mthfd2</i>	Methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	2.07	0.0008	+	-
<i>Psap</i>	Prosaposin	2.07	0.0147	+	-
<i>Esd</i>	Esterase D/formylglutathione hydrolase	2.06	0.0008	+	+
<i>Dhcr7</i>	7-Dehydrocholesterol reductase	2.06	0.002	+	-
<i>Alas2</i>	Aminolevulinic acid synthase 2, erythroid	2.03	0.0082	-	-
<i>Odc1</i>	Ornithine decarboxylase, structural 1	2.02	0.0008	-	+
<i>Bzrp</i>	Benzodiazepine receptor, peripheral	2.01	0.0008	+	-
<i>Gnaq</i>	Phosphatidylethanolamine binding protein	2.01	0.0287	+	-
<i>Myo10</i>	Myosin X	2.00	0.004	+	+
<i>Psph</i>	Phosphoserine phosphatase	2.00	0.0287	+	-

(Continued)

TABLE 11.3 (Continued)

Gene	Description	FC	<i>p</i>	PKA	PKC
<i>DOWNREGULATION</i>					
<i>Hmgb2</i>	High mobility group box 2	0.45	0.0008	+	–
<i>Ptn</i>	Pleiotrophin	0.45	0.0008	+	–
<i>Aldh1a1</i>	Aldehyde dehydrogenase family 1, subfamily A1	0.46	0.0008	+	+
<i>Pbx3</i>	Pre B-cell leukemia transcription factor 3	0.47	0.0008	+	+
<i>Slc12a2</i>	Solute carrier family 12, member 2	0.50	0.0008	–	–

Abbreviations: FC, fold change; PKA, protein kinase A; PKC, protein kinase C.

Modified data published by Schimmer BP, Cordova M, Cheng H, et al. *Endocrinology* 2006; 147: 2357–67.

fasciculata of 9-week-old male rats, using laser capture microdissection (LCM) and a rat cDNA microarray with 22,523 probes.²³ They found 235 and 231 transcripts upregulated more than two-fold in the zona glomerulosa and zona fasciculata, respectively, with an FDR less than 0.05 (Table 11.4). *Frzb*, which inhibits Wnt signaling, was differentially expressed in the zona fasciculata. In contrast, *Wnt4* showed a trend of upregulation in zona glomerulosa cells. Lack of functional WNT4/Wnt4 results in adrenal dysgenesis and compromised aldosterone synthesis in human patients with the female sex reversal and dysgenesis of kidneys, adrenals, and lungs (SERKAL) syndrome and knockout mice lacking *Wnt4*, respectively.^{24,25} It has been inferred that *Frzb* and *Wnt4* inversely modulate adrenal zonation during organogenesis and aldosterone synthesis after birth. Information on zone-specific changes in transcripts will help clarify the development and maintenance of adrenal zonation.

TRANSCRIPTOME ANALYSIS OF ADRENOCORTICAL CELLS IN A MOUSE MODEL OF HUMAN LIPOID ADRENAL HYPERPLASIA

Undoubtedly, mouse models of human disorders would help identify the pathophysiology of disorders and even help determine possible

approaches for potential new treatments. Human adrenal glands are not easily accessible and are rarely removed except in cancer, thus making a knockout animal with adrenal insufficiency particularly valuable. One such model, knockout mice lacking steroidogenic acute regulatory protein (StAR), has been described here in detail.

Congenital lipoid adrenal hyperplasia (lipoid CAH) is an autosomal recessive disease due to *STAR* gene mutations. StAR regulates the rate-limiting step of steroidogenesis to facilitate the translocation of newly synthesized cholesterol from the outer to inner mitochondrial membrane.^{17,26–28} Patients with lipoid CAH and knockout mice lacking StAR (*Star*^{−/−} mice) exhibit significant defects in steroid hormone biosynthesis and diffuse accumulation of cholesterol esters in the cytoplasm of adrenocortical or gonadal steroidogenic cells.^{29–33} Bose *et al.*²⁹ proposed the “two-hit” model, in which the first hit is loss of StAR-dependent steroidogenesis due to StAR deficiency and the second hit is loss of StAR-independent steroidogenesis caused by accumulated cholesterol esters, thus explaining the varying phenotypes among steroidogenic tissues. However, the molecular mechanisms underlying the cholesterol translocation by StAR and the abrogation of StAR-independent steroidogenesis by cholesterol deposits require clarification. Thus, the transcriptome of steroidogenic cells in the adrenal glands of *Star*^{−/−} mice

TABLE 11.4 Genes Significantly Upregulated in Rat Zona Glomerulosa or Zona Fasciculata by cDNA Microarray

Gene	Description	FC	p
ZONA GLOMERULOSA			
<i>Cyp11b2</i>	<i>Rattus norvegicus</i> cytochrome P450, family 11, subfamily B, polypeptide 2 (Cyp11b2), nuclear gene encoding mitochondrial protein, mRNA	214.2	0.00078
<i>Rgs4</i>	<i>Rattus norvegicus</i> regulator of G-protein signaling 4 (Rgs4), mRNA	68.4	0.00052
<i>Smoc2_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> SPARC related modular calcium binding 2 (predicted), transcript variant 2 (Smoc2_predicted), mRNA	49.3	0.00777
<i>Mia1</i>	PREDICTED: <i>Rattus norvegicus</i> melanoma inhibitory activity 1 (Mia1), mRNA	43.1	0.00031
<i>Dlk1</i>	<i>Rattus norvegicus</i> delta-like 1 homolog (<i>Drosophila</i>) (Dlk1), mRNA	38.3	0.00531
<i>Sstr2</i>	<i>Rattus norvegicus</i> somatostatin receptor 2 (Sstr2), mRNA	37.2	0.00852
<i>Cadps</i>	<i>Rattus norvegicus</i> Ca ²⁺ -dependent secretion activator (Cadps), mRNA	33.7	0.00483
<i>LOC311772</i>	PREDICTED: <i>Rattus norvegicus</i> similar to nidogen 2 (LOC311772), mRNA	33.6	0.00083
<i>Igsf1</i>	<i>Rattus norvegicus</i> immunoglobulin superfamily, member 1 (Igsf1), mRNA	32.9	0.00078
<i>LOC362564</i>	PREDICTED: <i>Rattus norvegicus</i> hypothetical LOC362564 (LOC362564), mRNA	31.1	0.00296
<i>Gpc3</i>	<i>Rattus norvegicus</i> glypican 3 (Gpc3), mRNA	30.6	0.01434
<i>Cpxm2_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> carboxypeptidase X 2 (M14 family) (predicted) (Cpxm2_predicted), mRNA	29.1	0.01163
<i>Atp10a</i>	PREDICTED: <i>Rattus norvegicus</i> ATPase, class V, type 10A, transcript variant 1 (Atp10a), mRNA	28.8	0.00118
<i>Postn_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> periostin, osteoblast specific factor (predicted) (Postn_predicted), mRNA	24.1	0.02619
<i>Boc_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> biregional cell adhesion molecule-related/downregulated by oncogenes (Cdon) binding protein (predicted) (Boc_predicted), mRNA	23.3	0.00604
<i>RGD1566317_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> similar to Tescalcin (predicted) (RGD1566317_predicted), mRNA	22.9	0.00149
<i>Ndn</i>	<i>Rattus norvegicus</i> necdin (Ndn), mRNA	19.9	0.00318
<i>Dpt_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> dermatopontin (predicted) (Dpt_predicted), mRNA	18.5	0.01768
<i>Kcnn2</i>	<i>Rattus norvegicus</i> potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2 (Kcnn2), mRNA	17.5	0.00582
<i>Rbp1</i>	<i>Rattus norvegicus</i> retinol binding protein 1, cellular (Rbp1), mRNA	17.4	0.00375
<i>Wfdc1</i>	<i>Rattus norvegicus</i> WAP four-disulfide core domain 1 (Wfdc1), mRNA	15.7	0.01163
<i>Acy3</i>	<i>Rattus norvegicus</i> aspartoacylase (aminoacylase) 3 (Acy3), mRNA	15.6	0.01682
<i>RGD1307506_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> similar to RIKEN cDNA 2310016C16 (predicted) (RGD1307506_predicted), mRNA	15.1	0.00299
<i>Ptgis</i>	<i>Rattus norvegicus</i> prostaglandin I ₂ (prostacyclin) synthase (Ptgis), mRNA	14.9	0.01147

(Continued)

TABLE 11.4 (Continued)

Gene	Description	FC	p
<i>Reck_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> reversion-inducing-cysteine-rich protein with kazal motifs (predicted) (<i>Reck_predicted</i>), mRNA	14.5	0.01353
<i>RGD1564008_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> similar to dapper 1 (predicted) (<i>RGD1564008_predicted</i>), mRNA	13.2	0.00483
<i>Nr0b1</i>	<i>Rattus norvegicus</i> nuclear receptor subfamily 0, group B, member 1 (<i>Nr0b1</i>), mRNA	12.7	0.01434
<i>Fmod</i>	<i>Rattus norvegicus</i> fibromodulin (<i>Fmod</i>), mRNA	12.6	0.02731
<i>Pde2a</i>	<i>Rattus norvegicus</i> phosphodiesterase 2A, cGMP-stimulated (<i>Pde2a</i>), mRNA	12.6	0.00184
<i>Dab2</i>	<i>Rattus norvegicus</i> disabled homolog 2, mitogen-responsive phosphoprotein (<i>Drosophila</i>) (<i>Dab2</i>), mRNA	12.4	0.00420
ZONA FASCICULATA			
<i>LOC363306</i>	PREDICTED: <i>Rattus norvegicus</i> similar to RIKEN cDNA 4930555G01 (<i>LOC363306</i>), mRNA	19.30	0.01525
<i>Ddah1</i>	<i>Rattus norvegicus</i> dimethylarginine dimethylaminohydrolase 1 (<i>Ddah1</i>), mRNA	16.21	0.00369
<i>LOC498373</i>	PREDICTED: <i>Rattus norvegicus</i> similar to RIKEN cDNA 1700001E04 (<i>LOC498373</i>), mRNA	15.55	0.01342
<i>Cidea_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> cell death-inducing DNA fragmentation factor, alpha subunit-like effector A (predicted) (<i>Cidea_predicted</i>), mRNA	15.54	0.00176
<i>Hpx</i>	<i>Rattus norvegicus</i> hemopexin (<i>Hpx</i>), mRNA	15.43	0.01921
<i>Hamp</i>	<i>Rattus norvegicus</i> hepcidin antimicrobial peptide (<i>Hamp</i>), mRNA	15.32	0.03461
<i>RGD1562717_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> similar to ABI gene family, member 3 (NESH) binding protein (predicted) (<i>RGD1562717_predicted</i>), mRNA	14.90	0.00572
<i>Cyp4f4</i>	<i>Rattus norvegicus</i> cytochrome P450, family 4, subfamily f, polypeptide 4 (<i>Cyp4f4</i>), mRNA	14.60	0.03031
<i>Serpina11</i>	<i>Rattus norvegicus</i> serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 11 (<i>Serpina11</i>), mRNA	13.03	0.00670
<i>LOC501497</i>	PREDICTED: <i>Rattus norvegicus</i> <i>LOC501497</i> (<i>LOC501497</i>), mRNA	11.73	0.00630
<i>Fabp6</i>	<i>Rattus norvegicus</i> fatty acid binding protein 6, ileal (gastrotropin) (<i>Fabp6</i>), mRNA	11.35	0.02633
<i>Nkx6-2_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> NK6 transcription factor related, locus 2 (<i>Drosophila</i>) (predicted) (<i>Nkx6-2_predicted</i>), mRNA	10.04	0.00385
<i>RGD1560609_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> similar to Vanin-3 (predicted) (<i>RGD1560609_predicted</i>), mRNA	9.79	0.03668
<i>Frzb</i>	PREDICTED: <i>Rattus norvegicus</i> frizzled-related protein (<i>Frzb</i>), mRNA	9.45	0.00565
<i>LOC363060</i>	<i>Rattus norvegicus</i> similar to RIKEN cDNA 1600029D21 (<i>LOC363060</i>), mRNA	9.31	0.03191
<i>Ephb6</i>	PREDICTED: <i>Rattus norvegicus</i> Eph receptor B6 (<i>Ephb6</i>), mRNA	9.07	0.00770
<i>Fbxo17</i>	<i>Rattus norvegicus</i> F-box only protein 17 (<i>Fbxo17</i>), mRNA	9.03	0.00483

(Continued)

TABLE 11.4 (Continued)

Gene	Description	FC	<i>p</i>
<i>Rab33a_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> RAB33A, member of RAS oncogene family (predicted) (<i>Rab33a_predicted</i>), mRNA	8.99	0.01338
<i>Hhex</i>	<i>Rattus norvegicus</i> hematopoietically expressed homeobox (<i>Hhex</i>), mRNA	8.96	0.02417
<i>Vnn1</i>	<i>Rattus norvegicus</i> vanin 1 (<i>Vnn1</i>), mRNA	8.80	0.00777
<i>Hsd11b2</i>	<i>Rattus norvegicus</i> hydroxysteroid 11-beta dehydrogenase 2 (<i>Hsd11b2</i>), mRNA	8.26	0.00589
<i>Mt1a</i>	<i>Rattus norvegicus</i> metallothionein 1a (<i>Mt1a</i>), mRNA	8.17	0.00876
<i>Macro1</i>	<i>Rattus norvegicus</i> MACRO domain containing 1 (<i>Macro1</i>), mRNA	7.99	0.00247
<i>Edg4_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> endothelial differentiation, lysophosphatidic acid G protein-coupled receptor 4 (predicted) (<i>Edg4_predicted</i>), mRNA	7.88	0.00483
<i>Dhcr7</i>	<i>Rattus norvegicus</i> 7-dehydrocholesterol reductase (<i>Dhcr7</i>), mRNA	7.15	0.00486
<i>LOC681153</i>	PREDICTED: <i>Rattus norvegicus</i> hypothetical protein LOC681153 (<i>LOC681153</i>), mRNA	6.96	0.03839
<i>Arih1</i>	<i>Rattus norvegicus</i> ariadne ubiquitin-conjugating enzyme E2 binding protein homolog 1 (<i>Drosophila</i>) (<i>Arih1</i>), mRNA	6.85	0.01501
<i>F11r</i>	<i>Rattus norvegicus</i> F11 receptor (<i>F11r</i>), mRNA	6.76	0.01682
<i>Comtd1_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> catechol-O-methyltransferase domain containing 1 (predicted) (<i>Comtd1_predicted</i>), mRNA	6.70	0.02182
<i>Amid_predicted</i>	PREDICTED: <i>Rattus norvegicus</i> apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death (predicted) (<i>Amid_predicted</i>), mRNA	6.49	0.04764

Abbreviation: FC, fold change.

Modified data published by Nishimoto K, Rigsby CS, Wang T, et al. *Endocrinology* 2012; 153: 1755–63.

could help identify related biological pathways that may help elucidate the pathophysiology of StAR deficiency. The following subsection is a review of findings published previously.³⁴

Targeted Expression of eGFP in Adrenocortical Cells

In order to collect pure adrenocortical cells, Ishii *et al.* generated transgenic mice carrying a bacterial artificial chromosome (BAC) transgene targeting eGFP to steroidogenic cells under the control of endogenous regulatory sequences from the mouse *Star* gene (StAR/eGFP mice).³⁴ For transgene construction,

eGFP cDNA was inserted into the coding region of *Star* in a BAC clone containing the mouse *Star* gene with 47 kb of the 5'-flanking region and 62 kb of the 3'-flanking regions (StAR/eGFP BAC) (Figure 11.2A). Anti-eGFP antibody immunoreactivity and intrinsic eGFP fluorescence were observed in the adrenocortical cells, the interstitial cells of testes, and the theca cells, luteal cells, and stromal cells of ovaries from mice carrying the StAR/eGFP BAC transgene (Figure 11.2B), consistent with the endogenous StAR expression. The adrenal glands were dissected from wild type (*Star*^{+/+}) embryos or *Star*^{-/-} embryos with the StAR/eGFP BAC transgene at embryonic day (E) 17.5 or E18.5. Single cell

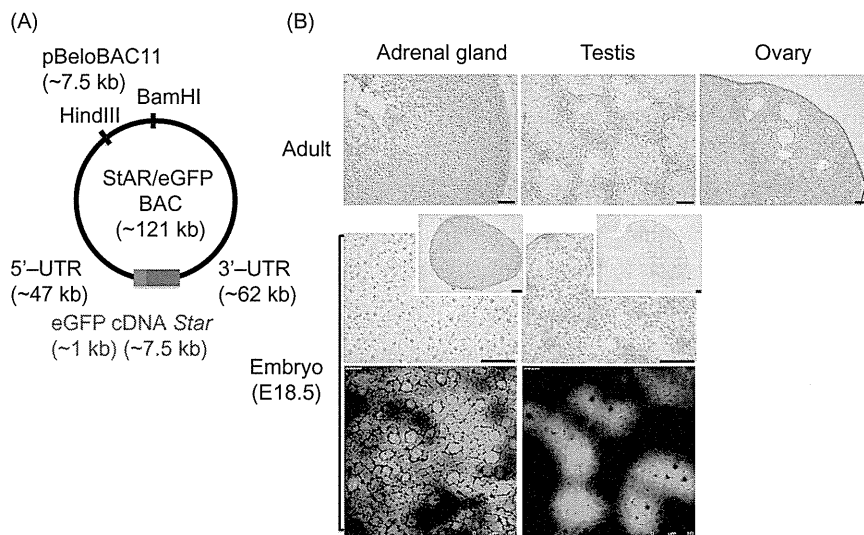


FIGURE 11.2 (A) Structure of the StAR/eGFP bacterial artificial chromosome (BAC) transgene. (B) Expression of the StAR/eGFP BAC transgene in adrenal glands and gonads from adults (top) or embryos at E18.5 (middle and bottom). Pictures in the top and middle show immunohistochemical staining for GFP expression in bright field lighting; those in the bottom demonstrate GFP fluorescence in dark field lighting. Each smaller picture in the middle shows a lower magnification view of the larger picture. Scale bars are 100 μm .

suspensions were prepared by enzymatic dissociation. eGFP-positive cells were selectively isolated by fluorescence-activated cell (FAC) sorting. Cell suspensions from the adrenal glands of *Star*^{+/+} or *Star*^{-/-} mice carrying no StAR/eGFP transgene were used as negative controls in FAC sorting to exclude the possibility of macrophage contamination due to autofluorescence.³⁵ StAR/eGFP mice provide a useful strategy for selective purification of StAR-expressing steroidogenic cells from the adrenal glands.

Differentially Regulated Genes in Mouse cDNA Microarrays

Ishii *et al.* determined the transcriptome of the adrenocortical cells of *Star*^{+/+} or *Star*^{-/-} mice using a Whole Mouse Genome Microarray G4122F, Cy3-labeled cRNAs (One-Color Quick Amp Labeling kit), and a Gene

Array Scanner (all from Agilent Technologies, Inc., Palo Alto, CA).³⁴ The data were analyzed using the Gene Spring GX 11.5.1 software (Agilent Technologies, Inc.). The difference in gene expression was assessed by averaging the normalized values and performing a pairwise analysis between *Star*^{+/+} mice and *Star*^{-/-} mice. DEGs were defined as having a gene expression ratio of ≥ 2 -fold and a *p* value evaluated by FDR < 0.2 .¹² The transcriptome analysis identified 1973 DEGs (1206 upregulated and 767 downregulated) between *Star*^{+/+} and *Star*^{-/-} mice (Figure 11.1).

The expression levels of the selected genes were validated by quantitative polymerase chain reaction (qPCR) using additional samples of FAC-sorted cells from the adrenal glands at E17.5 or E18.5. The relative gene expression in *Star*^{+/+} and *Star*^{-/-} mice was calculated using the ddCt method and examined using Student's *t* test, with a *p* value < 0.05 considered significant. The transcriptome

TABLE 11.5 Genes Differentially Expressed in Knockout Mice Lacking StAR Compared with those in Wild-Type Mice by Quantitative Real-Time PCR

Functional Category	Gene	Description	FC (Mean \pm SE)
Steroid hormone biosynthesis	<i>Nr5a1</i>	Nuclear receptor subfamily 5, group A, member 1	0.68 \pm 0.52
	<i>Mc2r</i>	Melanocortin 2 receptor	0.29 \pm 0.18
	<i>Mrap</i>	Melanocortin 2 receptor accessory protein	0.26 \pm 0.51
	<i>Star</i>	Steroidogenic acute regulatory protein	0.02 \pm 0.01*
	<i>Cyp11a1</i>	Cytochrome P450, family 11, subfamily a, polypeptide 1	0.63 \pm 0.48
	<i>Fdx1</i>	Ferredoxin 1	0.63 \pm 0.69
	<i>Fdxr</i>	Ferredoxin reductase	0.69 \pm 0.56
	<i>Hsd3b1</i>	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	0.51 \pm 0.53
	<i>Agtr1a</i>	Angiotensin II receptor, type 1a	0.76 \pm 0.58
	<i>Ldlr</i>	Low-density lipoprotein receptor	1.58 \pm 1.19
	<i>Scarb1</i>	Scavenger receptor class B, member 1	1.39 \pm 2.91
	<i>Hmgcr</i>	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase	0.77 \pm 0.31
	<i>Lipe</i>	Lipase, hormone sensitive	0.93 \pm 0.45
	Cholesterol biosynthesis and influx	<i>Npc2</i>	Niemann Pick type C2
<i>Stard3</i>		START domain containing 3	4.69 \pm 3.15*
<i>Bzrpl1</i>		Benzodiazapine receptor, peripheral-like 1	0.82 \pm 0.94
<i>Srebf1</i>		Sterol regulatory element binding factor 1	1.9 \pm 0.93
<i>Acat2</i>		Acetyl-Coenzyme A acetyltransferase 2	1.19 \pm 1.08
<i>Nr1h2</i>		Nuclear receptor subfamily 1, group H, member 2	3.04 \pm 1.21*
<i>Nr1h3</i>		Nuclear receptor subfamily 1, group H, member 3	7.76 \pm 1.38*
<i>Abca1</i>		ATP-binding cassette, sub-family A (ABC1), member 1	2.92 \pm 1.59*
Cholesterol metabolism and efflux	<i>Abcg1</i>	ATP-binding cassette, sub-family G (WHITE), member 1	5.69 \pm 3.1*
	<i>Abcb1a</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	0.37 \pm 0.93
	<i>Dhcr24</i>	24-Dehydrocholesterol reductase	0.54 \pm 0.62
	<i>ch25h</i>	Cholesterol 25-hydroxylase	0.57 \pm 1.69
	<i>Wt1</i>	Wilms tumor homolog	1.83 \pm 1.26
Adrenocortical development	<i>Nr0b1</i>	Nuclear receptor subfamily 0, group B, member 1	0.09 \pm 0.10*
	<i>Shh</i>	Sonic hedgehog	0.02 \pm 0.02*
	<i>Wnt4</i>	Wingless-related MMTV integration site 4	0.08 \pm 0.06*

(Continued)

TABLE 11.5 (Continued)

Functional Category	Gene	Description	FC (Mean \pm SE)
	<i>Cd86</i>	CD86 antigen	6.44 \pm 3.39*
	<i>Cd36</i>	CD36 antigen	3.29 \pm 2.03*
	<i>Cd51</i>	CD5 antigen-like	130.95 \pm 273.32*
	<i>Spp1</i>	Secreted phosphoprotein 1	259.15 \pm 133.85*
	<i>Ccl5</i>	Chemokine (C-C motif) ligand 5	113.14 \pm 144.27*
	<i>Cxcl9</i>	Chemokine (C-X-C motif) ligand 9	0.28 \pm 1.23
	<i>Clec7a</i>	C-type lectin domain family 7, member a	100.69 \pm 65.1*
Inflammatory response	<i>H2-Aa</i>	Histocompatibility 2, class II antigen A, alpha	36.41 \pm 147.76
	<i>H2-Ab1</i>	Histocompatibility 2, class II antigen A, beta 1	24.29 \pm 70.12
	<i>H2-Ea</i>	Histocompatibility 2, class II antigen E alpha	37.09 \pm 475.29
	<i>Tlr1</i>	Toll-like receptor 1	4.12 \pm 3.97
	<i>Tlr2</i>	Toll-like receptor 2	2.82 \pm 2.31
	<i>Tlr4</i>	Toll-like receptor 4	2.08 \pm 1.24
	<i>Tlr6</i>	Toll-like receptor 6	8.2 \pm 8.33
	<i>Tlr7</i>	Toll-like receptor 7	4.59 \pm 3.41

Asterisks (*) indicate statistically significant change ($p < 0.05$).

Modified data published by Ishii T, Mitsui T, Suzuki S, et al. *Endocrinology* 2012; 153: 2714–23.

analysis and subsequent qPCR validation did not show significant differences between *Star*^{+/+} and *Star*^{-/-} mice in the expression of genes related to steroid hormone biosynthesis, other than *Star*, or cholesterol biosynthesis and influx. However, significant upregulation of the genes involved in cholesterol efflux, such as those encoding liver X receptors (LXRs) and adenosine triphosphate (ATP)-binding cassette, was observed; significant downregulation of genes involved in adrenocortical development, such as dosage-sensitive sex reversal-adrenal hypoplasia critical region on X chromosome 1 (*Dax-1*; officially *Nr0b1*), wingless-related MMTV integration site 4 (*Wnt4*) and sonic hedgehog (*Shh*), was also

observed (Table 11.5). These data reflect the pathophysiological features of adrenocortical cells upon StAR deficiency.

Pathway Analysis

Ishii *et al.* performed pathway analysis through IPA and NextBio.³⁴ IPA returned a list of canonical pathways most relevant to the transcriptome data set by a right- or two-tailed Fisher's exact probability test.¹⁶ IPA identified significant associations between the DEGs and the canonical pathway of inflammatory or immune response, including dendritic cell maturation, antigen presentation pathway, allograft rejection

TABLE 11.6 Canonical Pathways Significantly Altered in Knockout Mice Lacking StAR Compared with those in Wild-Type Mice by Ingenuity Pathway Analysis

Canonical Pathway	<i>p</i>	Ratio
Dendritic cell maturation	2.13796E-10	2.27E-01
Antigen presentation pathway	1.41254E-09	3.72E-01
Allograft rejection signaling	1.62181E-08	2.00E-01
Role of NFAT in regulation of the immune response	1.90546E-08	2.21E-01
Altered T cell and B cell signaling in rheumatoid arthritis	2.51189E-08	2.86E-01
Graft-versus-host disease signaling	7.07946E-08	3.54E-01
PKC θ signaling in T lymphocytes	8.91251E-08	2.41E-01
Communication between innate and adaptive immune cells	0.0000001	2.23E-01
Systemic lupus erythematosus signaling	2.0893E-07	1.79E-01
CD28 signaling in T helper cells	2.51189E-07	2.48E-01
OX40 signaling pathway	6.76083E-07	2.04E-01
Autoimmune thyroid disease signaling	7.94328E-07	2.54E-01
TREM1 signaling	8.31764E-07	3.16E-01
Natural killer cell signaling	9.12011E-07	2.55E-01
B cell receptor signaling	1.62181E-06	2.33E-01
IL-4 signaling	1.99526E-06	3.00E-01
Fc γ RIIB signaling in b lymphocytes	2.51189E-06	3.02E-01
iCOS-iCOSL signaling in T helper cells	3.46737E-06	2.35E-01
Atherosclerosis signaling	4.36516E-06	2.40E-01
Glycosaminoglycan degradation	5.7544E-06	4.44E-01
Cytotoxic T lymphocyte-mediated apoptosis of target cells	1.02329E-05	1.88E-01
B cell development	1.54882E-05	2.97E-01
Fc γ receptor-mediated phagocytosis in macrophages and monocytes	1.77828E-05	2.53E-01
T helper cell differentiation	2.39883E-05	2.68E-01
Role of pattern recognition receptors in recognition of bacteria and viruses	3.16228E-05	2.56E-01
PI3K signaling in B lymphocytes	3.46737E-05	2.13E-01
Crosstalk between dendritic cells and natural killer cells	5.49541E-05	2.17E-01
Type I diabetes mellitus signaling	5.7544E-05	2.14E-01
LXR/RXR activation	6.76083E-05	2.41E-01
Leukocyte extravasation signaling	7.24436E-05	1.91E-01

(Continued)

TABLE 11.6 (Continued)

Canonical Pathway	<i>p</i>	Ratio
IL-8 signaling	8.31764E-05	1.94E-01
Production of nitric oxide and reactive oxygen species in macrophages	0.000147911	1.98E-01
IL-10 signaling	0.000165959	2.50E-01
Role of BRCA1 in DNA damage response	0.000165959	2.71E-01
CTLA4 signaling in cytotoxic T lymphocytes	0.000190546	2.16E-01
IL-12 signaling and production in macrophages	0.000190546	1.98E-01
T cell receptor signaling	0.000436516	2.14E-01
fMLP signaling in neutrophils	0.000489779	1.95E-01
Sphingolipid metabolism	0.000537032	2.14E-01
NF- κ B activation by viruses	0.000630957	2.28E-01
Renin-angiotensin signaling	0.000977237	2.02E-01
NF- κ B signaling	0.00128825	1.71E-01
Role of JAK1 and JAK3 in γ c cytokine signaling	0.001513561	2.27E-01
MSP-RON signaling pathway	0.001584893	2.50E-01
Phospholipase C signaling	0.001659587	1.53E-01
Primary immunodeficiency signaling	0.001778279	1.75E-01
Erythropoietin signaling	0.001778279	2.16E-01
Virus entry via endocytic pathways	0.001862087	2.04E-01
Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis	0.002511886	1.41E-01
Phospholipid degradation	0.002754229	2.10E-01

p values are calculated by Fischer's exact probability test.

Ratios indicate the number of differentially expressed genes by total number of genes that map to the pathway.

signaling, communication between innate and adaptive immune cells, and interleukin (IL)-4 signaling (Table 11.6). NextBio identified a list of biogroups or cell types most significantly correlated with the transcriptome data set by Fisher's exact probability test with a multiple comparison test.¹⁵ The NextBio analysis identified immune response as the most significant biogroup. The NextBio analysis identified macrophages

from the peripheral tissues of C57BL/6 mice, microglial cells from C57BL/6 mice, and follicular dendritic cells from the lymph nodes of BALB/cAnNCrIcrIj mice as the most significant cell types positively correlated with the DEGs (Table 11.7). Both pathway analyses consistently indicated that the inflammatory or immune response was significantly altered in the adrenocortical cells of *Star*^{-/-} mice.

TABLE 11.7 Cell types Significantly Altered in Knockout Mice Lacking StAR Compared with those in Wild-Type Mice by NextBio

Cell Type	Body System	Score	Correlation	<i>p</i>
Macrophage of peripheral tissue of C57BL/6 strain	Immune	263.88	+	2.50E-115
Microglial cell of C57BL/6 strain	Immune	255.83	+	7.8E-112
Dendritic cell follicular of lymph node of BALB/cAnNCrICrlj strain	Immune	238.97	+	1.60E-104
Macrophage of bone marrow of C57BL/6 strain	Immune	217.19	+	4.7E-95
Dendritic cell (CD11c+ CD11b_hi CD103-) of lung of BALB/cByJ strain	Immune	214.96	+	4.40E-94
Myeloid-derived suppressor cell (CD11b+) of spleen of BALB/c strain	Immune	208.02	+	4.6E-91
Macrophage of peritoneum of C57BL/6J strain	Immune	187.31	+	4.50E-82
Osteoclast of C57BL/6 strain	Musculoskeletal	180.83	+	2.9E-79
Dendritic cell of bone marrow of 129SvJae x C57BL/6 strain	Immune	173.11	+	6.60E-76
Macrophage of peritoneum of C57BL/6 strain	Immune	170.00	+	1.5E-74
Dendritic cell of bone marrow of C57BL/6 strain	Immune	160.33	+	2.30E-70
Monocyte (Ly6c_hi) of peripheral blood of C57BL/6 strain	Immune	156.61	+	9.60E-69
Macrophage (CD11c_int CD11b+ B220- CD4- CD8- CD169_hi F4/80+) of lymph node medulla of C57BL/6 strain	Immune	154.21	+	1.1E-67
Monocyte (Ly6c_lo) of peripheral blood of C57BL/6 strain	Immune	150.70	+	3.60E-66
Hematopoietic stem cell (CD45+ Sca1+) of muscle of C57BL/6 strain	Immune	146.86	+	1.7E-64
Macrophage (CD11b+ CD11c-) of lamina propria of small intestine of C57BL/6 strain	Immune	146.40	+	2.60E-64
Dendritic cell (CD11c+) of bone marrow of C57BL/6J strain	Immune	140.43	+	1E-61
Dendritic cell (myeloid CD8a-) of C57BL/6 strain	Immune	133.03	+	1.70E-58
Macrophage of mammary gland of FVB/N strain	Exocrine	130.98	+	1.3E-57

(Continued)

TABLE 11.7 (Continued)

Cell Type	Body System	Score	Correlation	<i>p</i>
Dendritic cell of bone marrow of BALB/c strain	Immune	127.28	+	5.30E-56
Macrophage (CD11b+ CD11c-) cell of spleen of C57BL/6 strain	Immune	126.92	+	7.6E-56
Neutrophil (lineage- c-kit- Gr1+ Mac1+) of bone marrow of C57BL/6 strain	Immune	125.64	+	2.70E-55
Monocyte (Mac1+) of peripheral blood of C57BL/6 strain	Immune	123.10	+	3.5E-54
Dendritic cell of lymph node of BALB/c strain	Immune	120.18	+	6.40E-53
Dendritic cell (CD11c_hi CD11b+ CD19- CD3- NK1.1- 120G8_lo) of spleen of C57BL/6 strain	Immune	114.76	+	1.5E-50
Dendritic cell (CD11c+ CD11b_lo CD103+) of lung of BALB/cByJ strain	Immune	112.02	+	2.30E-49
Dendritic cell (CD8- 33D1+ CD205- CD11c+) of spleen of C57BL/6 strain	Immune	110.27	+	1.3E-48
Dendritic cell of spleen of BALB/c strain	Immune	110.03	+	1.60E-48
Spermatogonial cell (Thy1-) of testis of C57BL/6 strain	Urogenital	108.87	-	5.2E-48
Dendritic cell (plasmacytoid CD11c_int CD19- CD3- NK1.1- 120G8_hi) of spleen of C57BL/6 strain	Immune	105.74	+	1.20E-46
B lymphocyte (B220+) of spleen of C57BL/6 strain	Immune	103.87	+	7.7E-46
Synovial fibroblast of knee joint of BALB/c strain	Musculoskeletal	103.11	+	1.70E-45
Dendritic cell (CD8+ 33D1- CD205+ CD11c+) of spleen of C57BL/6 strain	Immune	101.76	+	6.4E-45
Macrophage of bone marrow of BALB/c strain	Immune	101.51	+	8.20E-45
Myeloid-derived suppressor cell (CD11b+) of bone marrow of BALB/c strain	Immune	100.95	+	1.4E-44
Stem cell of spermatogonia of C57BL/6J strain	Urogenital	98.10	-	2.50E-43
Side population cell of tibialis anterior muscle of C57BL/6 strain	Musculoskeletal	93.86	+	1.7E-41
Cardiac progenitor cell of ROSA26 x Isl1 strain	Cardiovascular	91.09	-	2.80E-40

(Continued)

TABLE 11.7 (Continued)

Cell Type	Body System	Score	Correlation	<i>p</i>
Type II cell of lung of ICR10 strain	Respiratory	87.77	+	7.7E-39
Dendritic cell (IFN-producing killer) of lymph node of BALB/c strain	Immune	87.54	+	9.60E-39
Dendritic cell (CD11c+) of lamina propria of C57BL/6 strain	Immune	87.03	+	1.6E-38
B lymphocyte (B220+ CD19+ IgM+) of spleen of C57BL/6 strain	Immune	85.93	+	4.80E-38
Dendritic cell of Peyer's patch of BALB/c strain	Immune	84.02	+	3.2E-37
Mesenchymal cell of proximal forelimb of CD-1 strain	Integumentary	83.27	-	6.90E-37
Granulocyte (lineage- Gr-1+ clone7.4+) of bone marrow of C57BL/6 strain	Immune	82.65	+	1.3E-36
T lymphocyte (CD4+) of undetermined strain	Immune	82.12	+	2.20E-36
Dendritic cell (CD8-) of spleen of C57BL/6 strain	Immune	81.97	+	2.5E-36
Granulosa cell of ovary of C57BL/6J x 129S5/SvEvBrd strain	Urogenital	81.23	-	5.30E-36
Mesenchymal cell of distal hindlimb of CD-1 strain	Integumentary	81.03	-	6.4E-36
Mesothelial cell (Mesothelin+) of liver of C57BL/6 strain	Digestive	80.84	+	7.80E-36

The correlation score represents the magnitude of correlation between gene expression profiles, computed with the Running Fisher algorithm, a non-parametric rank-based statistical approach. Symbols of + and - indicate positive and negative correlation, respectively. *p* values are calculated by Fischer's exact probability test.

Pathophysiological Hallmarks Identified by the Transcriptome Analysis of the Adrenocortical Cells of *Star*^{-/-} Mice

In the study by Ishii *et al.*, genes involved in the inflammatory or immune response, the LXR signaling, and the adrenocortical zonation show a significant positive correlation with the DEGs in *Star*^{-/-} mice-derived adrenocortical cells.³⁴ The results provide useful information

to elucidate the pathophysiology of adrenocortical cells in patients with lipoid CAH.

Inflammatory or Immune Response

The inflammatory or immune response may play a physiological role in adrenocortical cells, especially under stress conditions. Woods and Judd reported that IL-4 signaling inhibited the expression of ACTH-induced steroidogenic

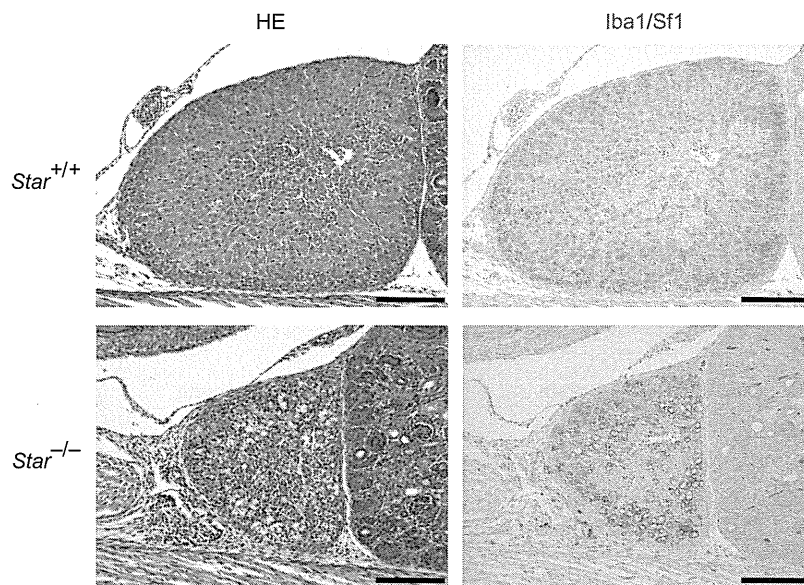


FIGURE 11.3 Immunohistochemistry for Iba1 (a macrophage marker) and *in situ* hybridization for Nr5a1 (a steroidogenic cell marker) in the adrenal glands at E18.5. Red and blue staining represents Iba1 immunoreactivity and Nr5a1 transcripts, respectively. Scale bars are 200 μm . HE, hematoxylin and eosin staining.

enzymes in bovine zona reticularis cells.³⁶ IL-4 signaling is one of the canonical pathways in which genes are significantly upregulated in *Star*^{+/+} mice (Table 11.6). The increased inflammatory or immune response, including IL-4 signaling, could be the second “hit” of the two-hit model for lipoid CAH, attenuating the signal transduction of melanocortin type 2 receptor under high levels of circulating ACTH. The actual role of the inflammatory or immune response in the adrenocortical cells of *Star*^{+/+} mice remains unclear.

Based on the results of transcriptome analysis, the expression pattern of a specific macrophage marker, Iba1, was examined in the adrenal glands by immunohistochemical analysis.³⁷ Fixed whole embryos at E17.5–18.5 were double-stained by immunohistochemistry and *in situ* hybridization for markers of macrophages (Iba1; officially *Aif1*, allograft inflammatory factor 1) and steroidogenic cells (*Sf1*; officially *Nr5a1*), respectively. Iba1 immunoreactivity was

detected in only several *Sf1*-negative resident macrophages within the adrenal cortex of *Star*^{+/+} mice, but in a larger number of *Sf1*-negative resident macrophages within the adrenal cortex of *Star*^{+/+} mice (Figure 11.3).

These findings suggest a link between the inflammatory or immune system and the adrenal glands. Adrenocortical cells produce various cytokines during acute stress.³⁸ Additionally, intra-adrenal macrophages influence the function of the adrenal gland in an autocrine or paracrine manner.³⁸ As shown in Figure 11.3, many macrophages infiltrated into the adrenal glands of *Star*^{+/+} mice.³⁴ *Star*^{+/+} mice-derived adrenocortical cells exhibited significantly higher mRNA levels of chemokine ligand 5 (Ccl5) and CD36 antigen (Cd36) than cells from *Star*^{+/+} mice.³⁴ Ccl5, an adipocytokine, recruits macrophages into adipose tissues.³⁹ CD36, a receptor for apoptosis inhibitor of macrophage (AIM), induces lipolysis and chemokine production in adipocytes.⁴⁰ These data suggest a reciprocal

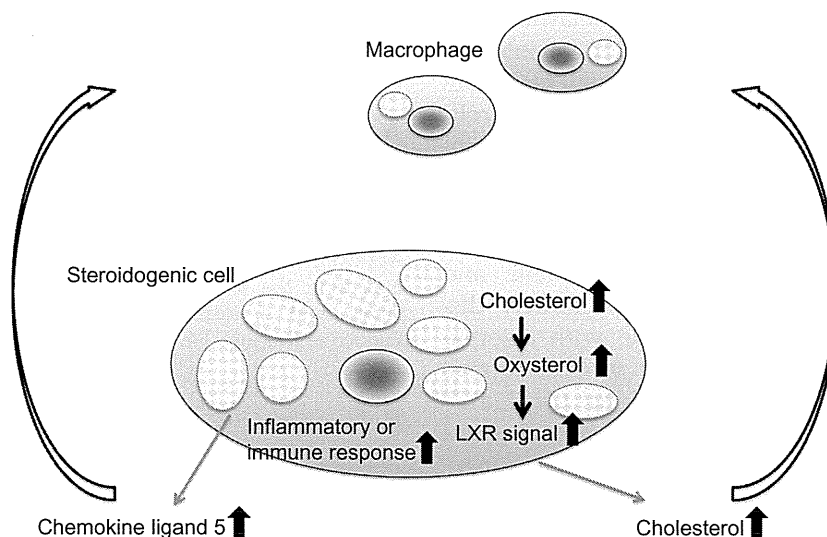


FIGURE 11.4 Schematic model of “adrenal remodeling” in *Star*^{-/-} mice. Upward arrow indicates an increased number of molecules or transcripts involved in the index pathways. Blue ovals indicate nuclei; yellow ovals indicate lipid droplets of cholesterol ester in the cytoplasm of adrenocortical cells or macrophages. LXR, liver X receptor.

interaction between adrenocortical cells and infiltrated macrophages upon disruption of StAR. The adrenal glands of *Star*^{-/-} mice may share some features of homeotic inflammation with white adipose tissue in patients with obesity and with vascular walls in those with atherosclerosis,^{41–43} which can be called “adrenal remodeling” (Figure 11.4).

LXR Signaling

Significant upregulation of LXRs α and β (Nr1h3 and Nr1h2, respectively) has been observed in *Star*^{-/-} mice-derived adrenocortical cells whose cytoplasm is filled with cholesterol esters.³⁴ Cummins *et al.* reported that knockout mice lacking LXRs α and β (*Lxr $\alpha\beta$* ^{-/-} mice) accumulated cholesterol esters.⁴⁴ Hence, it has been inferred that LXRs α and β play an important role in protecting adrenal glands from the accumulation of free cholesterol. Accumulated oxysterol intermediates could function as ligands for LXRs in the adrenocortical cells of *Star*^{-/-} mice and induce cholesterol efflux by increasing the expression of

ATP-binding cassettes (*Abc1* and *Abcg1*). Furthermore, enhanced LXR signaling could inhibit the expression of multiple steroidogenic genes, as reported in the H295R human adrenocortical cell line.⁴⁵ Cummins *et al.* showed increased adrenal steroidogenesis even with a decline of StAR expression in *Lxr $\alpha\beta$* ^{-/-} mice,⁴⁴ indicating a possible link between the repression of LXR signaling and the increase in StAR-independent steroidogenesis. These findings give additional credence to the role of LXRs in maintaining cholesterol homeostasis in adrenocortical cells and imply that LXR upregulation is relevant to the lost residual capacity for StAR-independent steroidogenesis as the second hit in the two-hit model for lipoid CAH.

Adrenocortical Zonation

Genes related to the transition from fetal to adult adrenocortical cells are significantly down-regulated in *Star*^{-/-} mice-derived adrenocortical cells.³⁴ The adult cortex ultimately develops from precursor cells in the fetal cortex, as shown

by a lineage tracing study by Morohashi's laboratory.⁴⁶ The adult cortex is then maintained through proliferation and the clonal replenishment of subcapsular progenitor or stem cells that probably move centripetally and differentiate into steroidogenic cells in the zona glomerulosa or fasciculata.^{22,47–49} Consistent with these ideas, the adrenal glands of *Star*^{-/-} mice are severely hypoplastic during embryonic and early neonatal stages, and are definitely disturbed during cortical zonation with disorganized mitochondria,³¹ similar to the adrenal glands of knockout mice lacking *Cyp11a1*.⁵⁰ Although StAR expression in adrenocortical progenitor or stem cells remains undetermined, decreased expression of the genes related to adrenocortical cell development in steroidogenic cells could directly or indirectly disturb normal proliferation and differentiation in the adrenal cortex of *Star*^{-/-} mice.

PERSPECTIVE

Transcriptome analysis allows for simultaneous investigation of an entire set of transcripts and permits parallel comparisons between different cells, such as healthy and diseased cells. As shown in this chapter, transcriptome analysis has helped identify significant alteration of gene expression associated with the inflammatory or immune response, the cholesterol efflux, and the adrenocortical zonation in the adrenocortical cells of *Star*^{-/-} mice. However, mRNA level may not always correlate with the biological function or phenotype of the cells. Biological function or phenotype is also affected by the corresponding protein level and modification. Bearing these limitations in mind, transcriptome analysis is a useful strategy for identifying hitherto undescribed functions or pathways to identify new pathophysiology or therapeutic approaches. If appropriately applied, global profiling of transcripts will provide an essential

tool for future research on the nature of adrenocortical cells.

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