

Expression of primary cilia-related genes in developing mouse gonads

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1	Expression of primary cilia markers in developing mouse gonads
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26 Abstract

Mechanisms governing differentiation of the bipotential gonad into the testes or ovaries are complex and still vague. The primary cilium is an organelle involved in cell signaling, which controls development of many organs, but the role of primary cillium in the sex determination and sexual differentiation of gonads is completely unknown. Here we studied the expression of marker genes of primary cilium in fetal mouse gonads, before, during and after sexual differentiation. We studied the expression of 175 genes considered to be the markers of primary cilia using microarray technique. 144 of these genes were ubiquitonously expressed in all studied cell types with no significant differences in expression level. Such high level of expression of markers of primary cilia in developing mouse gonads suggests that the primary cilia and/or primary cilia-related genes are important for development of both somatic and germline component of the gonads. Only 31 genes showed difference in the expression between different cell types, which suggests that they have different functions in the somatic and germ cells. These results justify further studies on the role of primary cilia and the primary cilia-related genes in gonad development.

51 Introduction

52 The testes and the ovaries develop from the bipotential gonads in the process of sexual differentiation. In the mouse, the gonadal primordia (genital ridges) appear just before 10.5th 53 54 day of embryonic life (E10.5) (Hu et al., 2013; Piprek et al., 2016). Between stage E10.5 and 55 E12.5, the sexually undifferentiated gonads start expressing the sex-determining genes (reviewed in Piprek et al., 2016). The fate of the gonad and its ultimate differentiation into the 56 57 testis or ovary depends on male or female sex-determining signalling pathways (reviewed in 58 Piprek, 2009a, 2009b). At stage E13.5, the mouse gonads are already sexually differentiated, 59 and their sex can be easily recognized under the microscope (Nel-Themaat et al., 2009). Developing gonads are composed of three basic cell types: i) supporting cells (Sertoli and 60 61 follicular cells), ii) interstitial/stromal cells, and iii) germ cells (Piprek et al., 2017, 2018). 62 Although a number of genes and signaling pathways (such as PDGF - platelet-derived growth 63 factor pathway, FGF - fibroblast growth factor pathway, WNT - wingless-type MMTV integration site family pathway, and Hedgehog pathway) involved in sex determination and 64 65 sexual differentiation of mouse gonad have been identified (reviewed in Piprek, 2009a, 66 2009b, Piprek 2010), the mechanisms directing bipotential gonad differentiation into the 67 testes or ovaries are very complex, and thus still require further studies. 68 Studies of the last decade identified the primary cilium as a key coordinator of 69 signaling during embryogenesis and organogenesis (Satir *et al.*, 2010). The primary cilium is 70 an immotile organelle present on the surface of large variety of eukaryotic cells. The primary 71 cilium contains peripheral doublets of microtubules and lacks central microtubules (9+0 72 axoneme pattern). The primary cilium disappears during cell division. Recently, it has been 73 shown that the primary cilium possesses various receptors and acts as "a cell's antenna",

which enables the cell to respond to various signaling molecules (reviewed in Wainwright *et*

75 *al.*, 2014). Recently, the markers of primary cilia, i.e. the genes important for primary cilium

formation and function, have been identified and characterized (Ishikawa et al., 2012). The 76 fact that primary cilium participates in receiving signals from PDGF, FGF, WNT and 77 Hedgehog pathways suggests that this organelle may be important for gonad development. 78 79 The expression of many primary cilia marker genes in developing mouse gonads suggests that 80 primary cilia or primary cilia-related mRNAs and proteins are present in three studied cell types and may play a role in differentiation of these cells and in sexual differentiation of 81 82 gonads. Very little is known about the presence of primary cilia in the gonads. Wainwright 83 and colleagues (2014) showed that the primary cilia are present in the somatic and germ cells 84 of fetal mouse gonads between stage E10.5 and E13.5 (Wainwright et al., 2014). However, from stage E13.5 onward only interstitial (Leydig and peritubular myoid) cells retain primary 85 86 cilia, and no primary cilia are present in the Sertoli or germ cells (Wainwright et al., 2014). 87 Also in the adult human testis, the primary cilia are only present in the Leydig and peritubular 88 myoid cells (Nygaard et al., 2015). In contrast, in pig developing testes, the primary cilia were 89 detected in Sertoli cells and interstitial cells, but not in the germ cells (Ou et al., 2014). 90 However, there are no studies on the role of primary cilium in sex determination and sexual 91 differentiation of gonads. Here we studied the expression of primary cilium markers in 92 supporting cells, interstitial/stromal cells and germ cells isolated from developing mouse 93 gonads at three developmental stages: E11.0 (sex determination period), E12.2 (the onset of 94 sexual differentiation), E13.8 (sexually differentiated gonads).

95

96 Results and Discussion

97 In 2012, Ishikawa and collaborators identified 195 primary cilia marker genes
98 expressed in mouse kidney cells (Ishikawa *et al.*, 2012). Here, we detected the expression of
99 175 of these genes in developing mouse gonads (Table 1,2,3,4). 144 of these genes were
100 ubiquitonously expressed in all studied cell types at E11.0, E12.2 and E13.8 with no

significant differences in expression level (Table 1), and only 31 genes showed difference in
the expression between cell types. Twenty five of these genes had different level of
expression between the somatic cells and the germ cells. Among these genes, 12 genes had
higher expression (Table 2), and 13 genes had lower expression in the germ cells (Table 3)
comparing to the somatic cells. Only 6 genes showed differences in the expression level
between supporting and interstitial/stromal cells (Table 4).

107 Among genetic markers of primary cilia ubiquitously expressed in developing mouse 108 gonads with no significant differences between cell types (fold change <1.5) were (144 genes) 109 for exaple: ADP-ribosylation factors (Arf), ADP ribosylation factor like GTPase 13B 110 (Arl13b), calumenin (Calu), chaperonin containing t-complex polypeptides (Cct), calponin 3 111 (Cnn3), exportin 2 (Cse11), cullin2 (Cul2), dynactin 2 (Dctn2), dynamin 1-like protein 112 (Dnm11), dynamin 2 (Dnm2), cytoplasmic dyneins (Dync), eukaryotic translation initiation 113 factors (Eif), intraflagellar transport proteins (Ift), importin 7 (Ipo7), kinesin family members 114 (Kif), nucleolin (Ncl), nardilysin (Nrd1), prostaglandin E synthase 3 (Ptges3), Ras-related 115 proteins (Rab), septins (Sept), and exportin 9 (Xpo7); Table 1. A function of ADP ribosylation 116 factor like GTPase 13B (Arl13b) is restricted to the primary cilia. This GTPase is localized in 117 the cilia and plays a role in cilia formation and their maintenance (Higginbotham et al., 2012). 118 Another genes restricted to the primary cilia are intraflagellar transport proteins Ift20, Ift88, 119 and *Ift172*. They are responsible for cilium biogenesis. The ubiquitous expression of these 120 genes in all cell types of developing mouse gonad suggests that all these cells possibly posses 121 primary cilia, at certain point of development. Indeed, the majority of above genes are known 122 to play other, more broader than cilia-related functions in a cell. This again indicates that the 123 functions of these genes in the developing gonad may be broader and not limited to the 124 primary cilia.

125	Among primary cilia genes expressed at significantly higher level (fold change ≥ 1.5)
126	in the germ cells than in somatic cells were (12 genes): arsenical pump-driving ATPase
127	(Asna1), protein diaphanous homolog 1 (Diap1), insulin-degrading enzyme (Ide), protein
128	phosphatase 2 regulatory subunit A beta (<i>Ppp2r1b</i>), peroxiredoxin 4 (<i>Prdx4</i>), 60S ribosomal
129	protein L12 (<i>Rpl12</i>), 60S acidic ribosomal protein P2 (<i>Rplp2</i>), ribosomal protein S12 (<i>Rps12</i>),
130	testis specific gene A14 (<i>Tsga14</i>), tubulin alpha-4A chain (<i>Tuba4a</i>), and exportin 5 (<i>Xpo5</i>);
131	Table 2. The Solute carrier family 2 member 1 (<i>Slc2a1</i>) was the only gene showing
132	significant difference in the expression level between XX and XY germ cells, with the higher
133	expression in the XY germ cells (Table 2), which suggests that this gene product may be
134	involved in differentiation of spermatogonia.
135	Among primary cilia genes expressed at significantly lower level (fold change ≥ 1.5) in
136	the germ cells than in somatic cells were (13 genes): ADP-ribosylation factor-like protein 3
137	(Arl3), Calcium/calmodulin-dependent protein kinase type II delta (Camk2d), calpain-2
138	catalytic subunit (Capn2), Erbb2 interacting protein (Erbb2ip), GNAS (guanine nucleotide
139	binding protein, alpha stimulating) complex locus (Gnas), isocitrate dehydrogenase 1 (Idh1),
140	peroxiredoxin 3 (Prdx3), protein tyrosine phosphatase non-receptor type 13 (Ptpn13), protein
141	transport protein Sec23A (Sec23a), septin 9 (Spet9), triosephosphate isomerase 1 (Tpi1),
142	tetratricopeptide repeat protein 30B (<i>Ttc30b</i>), and tubulin beta-2A chain (<i>Tubb2a</i>); Table 3.
143	Among marker genes showing significant differences in the expression level (fold
144	change \geq 1.5) between different type or sex of somatic cells were (6 genes): Ran GTPase-
145	activating protein 1 (Rangap1), protein transport protein Sec23B (Sec23b), syntrophin basic 2
146	(Sntb2), transmembrane protein 2 (Tmem2), and tubulin beta-3 chain (Tubb3). These genes
147	showed higher expression in XY than in XX supporting cells (Table 4). This suggests that
148	these genes may be involved in sex determination and/or sexual differentiation of supporting
149	cells. Another tubulin gene- tubulin beta-6 chain (Tubb6) had higher level of expression in the

interstitial/stromal cells than in the supporting cells (Table 4), which suggests its importancefor differentiation of the interstitial/stromal cells.

152 As mentioned above, the primary cilia were previously detected in the somatic cells of 153 developing mouse, pig, and human gonads but they were absent in the germ cells 154 (Wainwright et al., 2014; Nygaard et al., 2015; Ou et al., 2014). Presented here global 155 analysis of expression showed the expression of primary cilia markers in both somatic and 156 germ cells during the sex determination and sexual differentiation period of the gonad. 157 Further studies are necessary to establish if the germ cells in fetal mouse gonad possess 158 primary cilia at certain stage(s) and if there are any differences in the function of primary cilia 159 or primary cilia-related genes in different cell lines in differentiating gonads. Differences in 160 the expression of primary cilia markers between somatic and germ cells suggest that, indeed, 161 there is a difference in the function of primary cilia or primary cilia-related genes between 162 somatic and germ cells. Wainwright and colleagues (2014) showed that mice with mutation in Ift144 (intraflagellar transport gene 144) gene had abnormally large gonads and more testis 163 164 cords than control gonad (Wainwright et al., 2014). This indicate that indeed the primary cilia 165 marker gene is involved in gonad development. The transcriptome analysis presented in this 166 study creates a valuable data base, which will be crucial in further studies of the role of 167 primary cilia or their related genes in the development and/or differentiation of the gonads. 168

169 Materials and Methods

170 The study had been approved by the 1st Local Commission for Ethics in Experiments171 on Animals. Five transgenic mouse lines were used to isolate the supporting,

172 interstitial/stromal and germ cells as previously described (Piprek *et al.*, 2017). All individuals
173 were genotyped to define sex and the presence of transgene as previously described (Piprek *et al.*, 2017). Gonads from mouse fetuses were pooled accordingly to the sex and developmental

175	stage. The gonads were incubated in 250 μl 0.25% Trypsin–EDTA (Sigma, #T4049) at 37°C
176	for 5–10 minutes (Piprek et al., 2017). After tissue dissociation, the enzyme solution was
177	replaced with PBS. Cells were centrifuged, after which the cell pellet was resuspended in PBS
178	with Hoechst dye and incubated for 15 min. Fluorescence-activated cell sorting (FACS) was
179	used to segregate three cell types isolated from the gonads (Piprek et al., 2017). Total RNA
180	was isolated from each cell type and analyzed using microarray technique as previously
181	described (Piprek et al., 2017). Raw data were analyzed as previously described (Piprek et al.,
182	2017), and normalized data are available in Gene Expression Omnibus (accession number
183	GSE94806).
184	
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Table 1. Primary cilia markers expressed ubiquitously expressed in developing mouse gonads with no significant differences between cell types (fold change <1.5) between E11.0 and E13.8 [symbols: 'vestigial level of expression (1-6); + high level of expression (7-8); ++ strong expression (9-10); +++ very strong expression (11-12)].

Gene symbol	Gene name	Support	ing cells	Interstitial/s	stromal cells	Germ	n cells
-		XX	XY	XX	XY	XX	XY
Abce1	ATP-binding cassette sub-family E member 1	+++	+++	+++	+++	+++	+++
Acaca	Acetyl-Coenzyme A carboxylase alpha	+	+	+	+	+	+
Adpgk	ADP-dependent glucokinase	+	+	+	+	+	+
Aldh18a1	Aldehyde dehydrogenase 18 family, member A1						
Anp32e	Acidic leucine-rich phosphoprotein 32 member E	+++	+++	+++	+++	+++	+++
Ap2b1	AP-2 complex subunit beta-1	++	++	++	++	++	++
Arf4	ADP-ribosylation factor 4	+++	+++	+++	+++	+++	+++
Arf6	ADP-ribosylation factor 6	+++	+++	+++	+++	+++	+++
Arfgef1	ADP-ribosylation factor guanine nucleotide factor 1	+++	+++	+++	+++	+++	+++
Arhgap5	Rho GTPase activating protein 5	++	++	++	++	++	++
Arl13b	ADP-ribosylation factor-like protein 13B	++	++	++	++	++	++
Arpc3	Actin-related protein 2/3 complex subunit 3	++	++	++	++	++	++
Azi1	5-azacytidine-induced protein 1						
B230208H17	Putative GTP-binding protein Parf	++	++	++	++	++	++
Btf3	Basic transcription factor 3	++	++	++	++	++	++
Calu	Calumenin	+++	+++	+++	+++	+++	+++
Ccdc47	Coiled-coil domain-containing protein 47	+++	+++	+++	+++	+++	+++
Cct4	Chaperonin containing t-complex 1, subunit 4	++	++	++	++	++	++
Cct5	Chaperonin containing t-complex 1, subunit 5	+++	+++	+++	+++	+++	+++
Cct6a	Chaperonin containing t-complex 1, subunit 6a	+++	+++	+++	+++	+++	+++
Cct8	Chaperonin containing t-complex 1, subunit 8	+++	+++	+++	+++	+++	+++
Chmp4b	Charged multivesicular body protein 4b	++	++	++	++	++	++
Cluap1	Clusterin-associated protein 1	++	++	++	++	++	++
Cnn3	Calponin 3	++	++	++	++	++	++
Спр	2',3'-cyclic nucleotide 3' phosphodiesterase	•		•		•	•
Cog4	Conserved oligomeric Golgi complex subunit 4	+	+	+	+	+	+

Copb2	Coatomer subunit beta	+++	+++	+++	+++	+++	+++
Cse1l	Exportin 2	+++	+++	+++	+++	+++	+++
Cul2	Cullin 2	+	+	+	+	+	+
D630037F22	Broad-minded	+	+	+	+	+	+
Daam1	Disheveled-associated activator of morphogenesis 1	++	++	++	++	++	++
Dcdc2a	Doublecortin domain-containing protein 2a		•	·		•	
Dctn2	Dynactin 2	++	++	++	++	++	++
Dhx30	DEAH box polypeptide 30	+	+	+	+	+	+
Dnm1l	Dynamin-1-like protein	++	++	++	++	++	++
Dnm2	Dynamin 2	++	++	++	++	++	++
Drg1	Developmentally-regulated GTP-binding protein 1	+++	+++	+++	+++	+++	+++
Drg2	Developmentally-regulated GTP-binding protein 2	+	+	+	+	+	+
Dync1h1	Cytoplasmic dynein 1 heavy chain 1	++	++	++	++	++	++
Dync1li1	Cytoplasmic dynein 1 light intermediate chain 1	++	++	++	++	++	++
Dync2h1	Cytoplasmic dynein 2 heavy chain 1	+	+	+	+	+	+
Dync2li1	Cytoplasmic dynein 2 light intermediate chain 1	+	+	+	+	+	+
Edc4	Enhancer of mRNA-decapping protein 4				•	•	
Eef1d	Eukaryotic translation elongation factor 1 delta	++	++	++	++	++	++
Efcab7	EF-hand calcium-binding domain-containing protein 7	++	++	++	++	++	++
Eif2s2	Eukaryotic translation initiation factor 2 subunit 2	++	++	++	++	++	++
Eif3b	Eif3b protein	+++	+++	+++	+++	+++	+++
Eif3l	Eukaryotic translation initiation factor 3 subunit L	+++	+++	+++	+++	+++	+++
Eif4g1	Eukaryotic translation initiation factor 4, gamma 1	++	++	++	++	++	++
Eif4h	Eukaryotic translation initiation factor 4H	+++	+++	+++	+++	+++	+++
Eif5b	Eukaryotic translation initiation factor 5B	+++	+++	+++	+++	+++	+++
Epb4.1l2	Erythrocyte protein band 4.1-like 2	+++	+++	+++	+++	+++	+++
Eps15l1	Epidermal growth factor receptor substrate 15-like 1	+	+	+	+	+	+
Etfb	Electron transfer flavoprotein subunit beta	+++	+++	+++	+++	+++	+++
Fam114a2	Family with sequence similarity 114, member A2	++	++	++	++	++	++
Fam49b	Family with sequence similarity 49, member B	++	++	++	++	++	++
Flii	Flightless 1 homolog	+	+	+	+	+	+
G3bp1	Ras GTPase-activating protein-binding protein 1	++	++	++	++	++	++

Gart	Phosphoribosylglycinamide formyltransferase	++	++	++	++	++	++
Gbf1	Golgi-specific brefeldin A-resistance factor 1	++	++	++	++	++	++
Gtl3	Gene trap locus 3	++	++	++	++	++	++
Hars	Putative uncharacterized protein	++	++	++	++	++	++
Hspa1a	Heat shock protein 1A	+++	+++	+++	+++	+++	+++
Hspa4	Heat shock 70 kDa protein 4	+++	+++	+++	+++	+++	+++
Hspb1	Heat shock protein beta-1		•		·	•	
Hspb11	Putative uncharacterized protein	++	++	++	++	++	++
Hsph1	Heat shock protein 105 kDa	+++	+++	+++	+++	+++	+++
lft122	Intraflagellar transport protein 122 homolog	•	•		•	•	
lft140	intraflagellar transport 140	+	+	+	+	+	+
lft172	Intraflagellar transport protein 172 homolog	+	+	+	+	+	+
lft20	Intraflagellar transport protein 20 homolog	++	++	++	++	++	++
lft52	Intraflagellar transport protein 52 homolog	++	++	++	++	++	++
lft57	Intraflagellar transport protein 57 homolog	++	++	++	++	++	++
lft74	Intraflagellar transport protein 74 homolog	++	++	++	++	++	++
lft80	Intraflagellar transport protein 80 homolog	++	++	++	++	++	++
lft81	Intraflagellar transport protein 81 homolog	++	++	++	++	++	++
lft88	Putative uncharacterized protein	++	++	++	++	++	++
Inpp5e	Inositol polyphosphate-5-phosphatase E	•	•		•		•
Invs	Inversin	+	+	+	+	+	+
Ipo5	Importin 5	++	++	++	++	++	++
lpo7	Importin 7	++	++	++	++	++	++
Kif3a	Kinesin family member 3A	++	++	++	++	++	++
Kif3b	Kinesin family member 3B	+	+	+	+	+	+
Kifap3	Kinesin-associated protein 3	+	+	+	+	+	+
Lamb1	Laminin B1	++	++	++	++	++	++
Lca5	Leber congenital amaurosis 5	+	+	+	+	+	+
Lrrc40	Leucine-rich repeat-containing protein 40	++	++	++	++	++	++
Lrrc59	Leucine-rich repeat-containing protein 59	+	+	+	+	+	+
Macf1	Microtubule-actin cross-linking factor 1	++	++	++	++	++	++
Mcm4	DNA replication licensing factor MCM4	++	++	++	++	++	++

Mtap1s	Microtubule-associated protein 1S	+	+	+	+	+	+
Ncl	Nucleolin	+++	+++	+++	+++	+++	+++
Nek8	Serine/threonine-protein kinase Nek8						
Nme7	Nucleoside diphosphate kinase 7	++	++	++	++	++	++
Nphp3	Nephronophthisis 3						
Nrd1	Nardilysin	+++	+++	+++	+++	+++	+++
Nudcd1	NudC domain-containing protein 1	++	++	++	++	++	++
Ogdh	Oxoglutarate dehydrogenase	++	++	++	++	++	++
Osbpl3	Oxysterol binding protein-like 3				•		
Pa2g4	Proliferation-associated protein 2G4	+++	+++	+++	+++	+++	+++
Pak2	Serine/threonine-protein kinase PAK 2	++	++	++	++	++	++
Pdia3	Protein disulfide-isomerase A3	+++	+++	+++	+++	+++	+++
Pin1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	•					
Ррр2са	Protein phosphatase 2, catalytic subunit alpha	+++	+++	+++	+++	+++	+++
Prmt5	Protein arginine N-methyltransferase 5	++	++	++	++	++	++
Prom1	Prominin 1		•				
Psmb5	Proteasome subunit beta type-5	+++	+++	+++	+++	+++	+++
Psmc1	26S protease regulatory subunit 4	+++	+++	+++	+++	+++	+++
Psmd5	26S proteasome non-ATPase regulatory subunit 5	++	++	++	++	++	++
Ptges3	Prostaglandin E synthase 3	+++	+++	+++	+++	+++	+++
Rab10	Ras-related protein Rab10	+++	+++	+++	+++	+++	+++
Rab23	Ras-related protein Rab23	++	++	++	++	++	++
Rab8a	Ras-related protein Rab-8A	++	++	++	++	++	++
Rabl5	Rab-like protein 5	+	+	+	+	+	+
Ran	RAN, member RAS oncogene family	+++	+++	+++	+++	+++	+++
Ranbp1	Ran-specific GTPase-activating protein	+++	+++	+++	+++	+++	+++
Rpl4	60S ribosomal protein L4	+++	+++	+++	+++	+++	+++
Rps14	40S ribosomal protein S14	+++	+++	+++	+++	+++	+++
Rpsa	40S ribosomal protein SA	+	+	+	+	+	+
Ruvbl2	RuvB-like 2	++	++	++	++	++	++
Sars	Seryl-aminoacyl-tRNA synthetase	++	++	++	++	++	++
Sec24b	Sec24 related gene family, member B	++	++	++	++	++	++

Sept2	Septin 2	+++	+++	+++	+++	+++	+++	
Sept7	Septin 7	+++	+++	+++	+++	+++	+++	
Serbp1	Serpine1 mRNA binding protein 1	+++	+++	+++	+++	+++	+++	
Stk10	Serine/threonine-protein kinase 10	•	•	•	·	·	•	
Surf4	Surfeit locus protein 4	++	++	++	++	++	++	
Syncrip	Synaptotagmin binding RNA interacting protein	+++	+++	+++	+++	+++	+++	
Tpd52	Tumor protein D52		•		·		•	
Traf3ip1	TRAF3-interacting protein 1	+	+	+	+	+	+	
Ttc21b	Tetratricopeptide repeat domain 21B	+	+	+	+	+	+	
Ttc26	Tetratricopeptide repeat protein 26		·		•		•	
Tubb2b	Tubulin beta-2B chain	+	+	+	+	+	+	
Tubb5	Tubulin beta-5 chain	+++	+++	+++	+++	+++	+++	
Ube4b	Ubiquitin conjugation factor E4 B	++	++	++	++	++	++	
Usp14	Ubiquitin carboxyl-terminal hydrolase 14	+++	+++	+++	+++	+++	+++	
Wdr11	WD repeat domain 11	++	++	++	++	++	++	
Wdr19	WD repeat domain 19	+	+	+	+	+	+	
Wdr34	WD repeat domain 34	+	+	+	+	+	+	
Wdr35	WD repeat domain 35	+	+	+	+	+	+	
Wdr60	WD repeat-containing protein 60	+	+	+	+	+	+	
Хро7	Exportin 7	++	++	++	++	++	++	
1500003003	Uncharacterized protein	+	+	+	+	+	+	
1500003003 Uncharacterized protein + + + + + + + + + + + + + + + + + + +								

Table 2. Primary cilia markers expressed at higher level in the germ cells comparing to the gonadal somatic cells (fold change ≥ 1.5) between E11.0 and E13.8 [symbols: vestigial level of expression (1-6); + high level of expression (7-8); ++ strong expression (9-10); +++ very strong expression (11-12)].

Gene symbol	Gene name	Support	ing cells	Interstitial/s	stromal cells	Gern	n cells
		XX	XY	XX	XY	XX	XY
Asna1	Arsenical pump-driving ATPase	+	+	+	+	++	++
Diap1	Protein diaphanous homolog 1	+	+	+	+	++	++
Ide	Insulin-degrading enzyme	+	+	+	+	+++	+++
Ppp2r1b	Protein phosphatase 2, regulatory subunit A, beta	++	++	++	++	+++	+++
Prdx4	Peroxiredoxin 4	++	++	++	++	+++	+++
Rpl12	60S ribosomal protein L12	•		•	•	+	+
Rplp2	60S acidic ribosomal protein P2	++	++	++	++	+++	+++
Rps12	Ribosomal protein S12	+	+	+	+	+++	+++
Slc2a1	Solute carrier family 2, member 1	+	+	+	+	++	+++
Tsga14	Testis specific gene A14	·	•	•	•	++	++
Tuba4a	Tubulin alpha-4A chain					++	++
Хро5	Exportin 5	++	++	++	++	+++	+++

Table 3. Primary cilia markers expressed at lower level in the germ cells comparing to the gonadal somatic cells (fold change ≥ 1.5) between E11.0 and E13.8 [symbols: vestigial level of expression (1-6); + high level of expression (7-8); ++ strong expression (9-10); +++ very strong expression (11-12)].

Gene symbol	Gene name	Supporting cells		Interstitial/stromal cells		Germ cells	
_		XX	XY	XX	XY	XX	XY
Arl3	ADP-ribosylation factor-like protein 3	++	++	++	++	+	+
Camk2d	Calcium/calmodulin-dependent protein kinase II, delta	++	++	++	++	•	•
Capn2	Calpain-2 catalytic subunit	++	++	++	++	•	
Erbb2ip	Erbb2 interacting protein	+++	+++	+++	+++	++	++
Gnas	GNAS (guanine nucleotide binding protein)	++	++	++	++	+	+
ldh1	Isocitrate dehydrogenase 1	+++	+++	+++	+++	++	++
Prdx3	Peroxiredoxin 3	++	++	++	++	+	+
Ptpn13	Protein tyrosine phosphatase, non-receptor type 13	++	++	++	++	+	+
Sec23a	Protein transport protein Sec23A	++	++	++	++	+	+
Sept9	Septin 9	++	++	++	++	+	+
Tpi1	Triosephosphate isomerase	++	++	++	++	+	+
Ttc30b	Tetratricopeptide repeat protein 30B	++	++	++	++	+	+
Tubb2a	Tubulin beta-2A chain	+++	+++	+++	+++	+	+

Table 4. Primary cilia markers showing significant differences in the expression level (fold change ≥ 1.5) between different type or sex of somatic cells between E11.0 and E13.8 [symbols: vestigial level of expression (1-6); + high level of expression (7-8); ++ strong expression (9-10); +++ very strong expression (11-12)].

Gene symbol	Gene name	Supporting cells		Interstitial/stromal cells		Germ cells	
		XX	XY	XX	XY	XX	XY
Rangap1	Ran GTPase-activating protein 1	+	++	+	+	+	+
Sec23b	Protein transport protein Sec23B	+	++	+	+	+	+
Sntb2	Syntrophin, basic 2	+	++	+	+	+	+
Tmem2	Transmembrane protein 2	+	++	+	+	+	+
Tubb3	Tubulin beta-3 chain	•	++		•	•	
Tubb6	Tubulin beta-6 chain	•	•	++	++	•	