Int. J. Dev. Biol. 63: 615-621 (2019)
https://doi.org/10.1387/ijdb.190049rp

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Expression of primary cilia-related genes in developing mouse gonads

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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 the development of many organs, but the role of primary cilium in the sex determination and sexual differentiation of gonads is completely unknown. Here we studied the expression of genes involved in primary cilium formation and functioning in fetal mouse gonads, before, during and after sexual differentiation. We studied the expression of 175 primary cilia-related genes using microarray technique. 144 of these genes were ubiquitously expressed in all studied cell types with no significant differences in expression level. Such a high level of expression of primary cilia-related genes in developing mouse gonads suggests that the primary cilia and/or primary cilia-related genes are important for the development of both somatic and germline component of the gonads. Only 31 genes showed a difference in 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.

KEY WORDS: *gonad development, sex determination, ovary, testis, primary cilia*

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 day of embryonic life (E10.5) (Hu *et al*., 2013; Piprek *et al*., 2016). Between stage E10.5 and 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 testis or ovary depends on male or female sex-determining signaling pathways (reviewed in Piprek, 2009a, 2009b). At stage E13.5, the mouse gonads are already sexually differentiated, 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 follicular cells), ii) interstitial/stromal cells, and iii) germ cells (Piprek *et al*., 2017, 2018). Although a number of genes and signaling pathways (such as PDGF - platelet-derived growth factor pathway, FGF - fibroblast growth factor pathway, WNT - wingless-type MMTV integration site family pathway, and Hedgehog pathway) involved in sex determination and sexual differentiation of mouse gonad have been identified (reviewed in Piprek, 2009a,b, 2010), the mechanisms directing bipotential gonad differentiation into the testes or ovaries are very complex, and thus still require further studies.

Studies of the last decade identified the primary cilium as a key coordinator of signaling during embryogenesis and organogenesis (Satir *et al*., 2010). The primary cilium is an immotile organelle present on the surface of a large variety of eukaryotic cells. The primary cilium contains peripheral doublets of microtubules and lacks central microtubules (9+0 axoneme pattern). The primary cilium disappears during cell division. Recently, it has been 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 al*., 2014). Recently, the genes important for primary cilium formation and function,

Abbreviations used in this paper: FGF, fibroblast growth factor.

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Submitted: 27 March, 2019; *Accepted:* 1 August, 2019.

ISSN: Online 1696-3547, Print 0214-6282

have been identified and characterized (Ishikawa *et al.*, 2012). The fact that primary cilium participates in receiving signals from PDGF, FGF, WNT and Hedgehog pathways suggests that this organelle may be important for gonad development. The expression of many primary cilia-related genes in developing mouse gonads suggests that primary cilia or primary cilia-related mRNAs and proteins are present in three studied cell types and may play a role in the differentiation of these cells and in sexual differentiation of gonads. Very little is known about the presence of primary cilia in the gonads. Wainwright and colleagues (2014) showed that the primary cilia are present in the somatic and germ cells 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 cilia, and no primary cilia are present in the Sertoli or germ cells (Wainwright *et al.*, 2014). Also in the adult human testis, the primary cilia are only present in the Leydig and peritubular myoid cells (Nygaard *et al.*, 2015). In contrast, in pig developing testes, the primary cilia were detected in Sertoli cells and interstitial cells, but not in the germ cells (Ou *et al.*, 2014). However, there are no studies on the role of the primary cilium in sex determination and sexual differentiation of gonads. Here we studied the expression of primary cilium-related genes in supporting cells, interstitial/stromal cells and germ cells isolated from developing mouse gonads at three developmental stages: E11.0 (the sex determination period), E12.2 (the onset of sexual differentiation), E13.8 (sexually differentiated gonads), using microarray technique supported by qPCR.

Results and Discussion

In 2012, Ishikawa and collaborators identified 195 primary cilia-related genes expressed in mouse kidney cells (Ishikawa *et al.*, 2012). Here, we detected the expression of 175 of these genes in developing mouse gonads (Table 1,2,3,4). 144 of these genes were ubiquitously 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 the difference in the expression between cell types. Twenty-five of these genes had a different level of expression between the somatic cells and the germ cells. Among these genes, 12 genes had a 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). qPCR analysis of 8 genes expression confirmed results of microarray analysis (Fig. 1).

Among the primary cilia-related genes ubiquitously expressed in developing mouse gonads with no significant differences between cell types (fold change <1.5) were (144 genes) for example: ADP-ribosylation factors (*Arf*), ADP ribosylation factor like GTPase 13B (*Arl13b*), calumenin (*Calu*), chaperonin containing t-complex polypeptides (*Cct*), calponin 3 (*Cnn3*), exportin 2 (*Cse1l*), cullin2 (*Cul2*), dynactin 2 (*Dctn2*), dynamin 1-like protein (*Dnm1l*), dynamin 2 (*Dnm2*), cytoplasmic dyneins (*Dync*), eukaryotic translation initiation factors (*Eif*), intraflagellar transport proteins (*Ift*), importin 7 (*Ipo7*), kinesin family members (*Kif*), nucleolin (*Ncl*), nardilysin (*Nrd1*), prostaglandin E synthase 3 (*Ptges3*), Ras-related proteins (*Rab*), septins (*Sept*), and exportin 9 (*Xpo7*); Table 1. A function of ADP ribosylation factor like GTPase 13B (*Arl13b*) is restricted to the primary cilia. This GTPase is localized in the cilia and plays a role in cilia formation and their maintenance (Higginbotham *et al.*, 2012). Other genes important for the primary cilia are intraflagellar transport proteins *Ift20*, *Ift88*, and *Ift172*. They are responsible for cilium biogenesis. The ubiquitous expression of these genes in all cell types of developing mouse gonad suggests that, at certain point in development, all these cells contain primary cilia. Indeed, the majority of the above genes are known to play other, broader than cilia-related functions in a cell. This again indicates that the functions of these genes in the developing gonad may be broader and not limited to the primary cilia.

Among primary cilia-related genes expressed at significantly higher level (fold change ≥1.5) in the germ cells than in somatic cells were (12 genes): arsenical pump-driving ATPase (*Asna1*), protein diaphanous homolog 1 (*Diap1*), insulin-degrading enzyme (*Ide*), protein phosphatase 2 regulatory subunit A beta (*Ppp2r1b*), peroxiredoxin 4 (*Prdx4*), 60S ribosomal protein L12 (*Rpl12*), 60S acidic ribosomal protein P2 (*Rplp2*), ribosomal protein S12 (*Rps12*), testis-specific gene A14 (*Tsga14*), tubulin alpha-4A chain (*Tuba4a*), and exportin 5 (*Xpo5*); Table 2. The Solute carrier family 2 member 1 (*Slc2a1*) was the only gene showing a significant difference in the expression level between XX and XY germ cells, with the higher expression in the XY germ cells (Table 2), which suggests that this gene product may be involved in differentiation of spermatogonia.

Among primary cilia-related genes expressed at significantly lower level (fold change ≥1.5) in the germ cells than in somatic cells were (13 genes): ADP-ribosylation factor-like protein 3 (*Arl3*), Calcium/calmodulin-dependent protein kinase type II delta (*Camk2d*), calpain-2 catalytic subunit (*Capn2*), Erbb2 interacting protein (*Erbb2ip*), GNAS (guanine nucleotide-binding protein, alpha stimulating) complex locus (*Gnas*), isocitrate dehydrogenase 1 (*Idh1*), peroxiredoxin 3 (*Prdx3*), protein tyrosine phosphatase non-receptor type 13 (*Ptpn13*), protein transport protein Sec23A (*Sec23a*), septin 9 (*Spet9*), triosephosphate isomerase 1 (*Tpi1*), tetratricopeptide repeat protein 30B (*Ttc30b*), and tubulin beta-2A chain (*Tubb2a*); Table 3.

Among primary cilia-related genes showing significant differences in the expression level (fold change ≥1.5) between different type or sex of somatic cells were (6 genes): Ran GTPase-activating protein 1 (*Rangap1*), protein transport protein Sec23B (*Sec23b*), syntrophin basic 2 (*Sntb2*), transmembrane protein 2 (*Tmem2*), and tubulin beta-3 chain (*Tubb3*). These genes showed higher expression in XY than in XX supporting cells (Table 4). This suggests that these genes may be involved in sex determination and/or sexual differentiation of supporting cells. Another tubulin gene, tubulin beta-6 chain (*Tubb6*), had a higher level of expression in the interstitial/stromal cells than in the supporting cells (Table 4), which suggests its importance for differentiation of the interstitial/stromal cells.

As mentioned above, the primary cilia were previously detected in the somatic cells of developing mouse, pig, and human gonads but they were absent in the germ cells (Nygaard *et al.*, 2015; Ou *et al.*, 2014; Wainwright *et al.*, 2014). Presented here the global analysis of expression showed the expression of primary cilia-related genes in both somatic and germ cells during the sex determination and sexual differentiation period of the gonad. Further studies are necessary to establish if the germ cells in fetal mouse gonad possess primary cilia at a certain stage(s) and if there are any differences in the function of primary cilia or

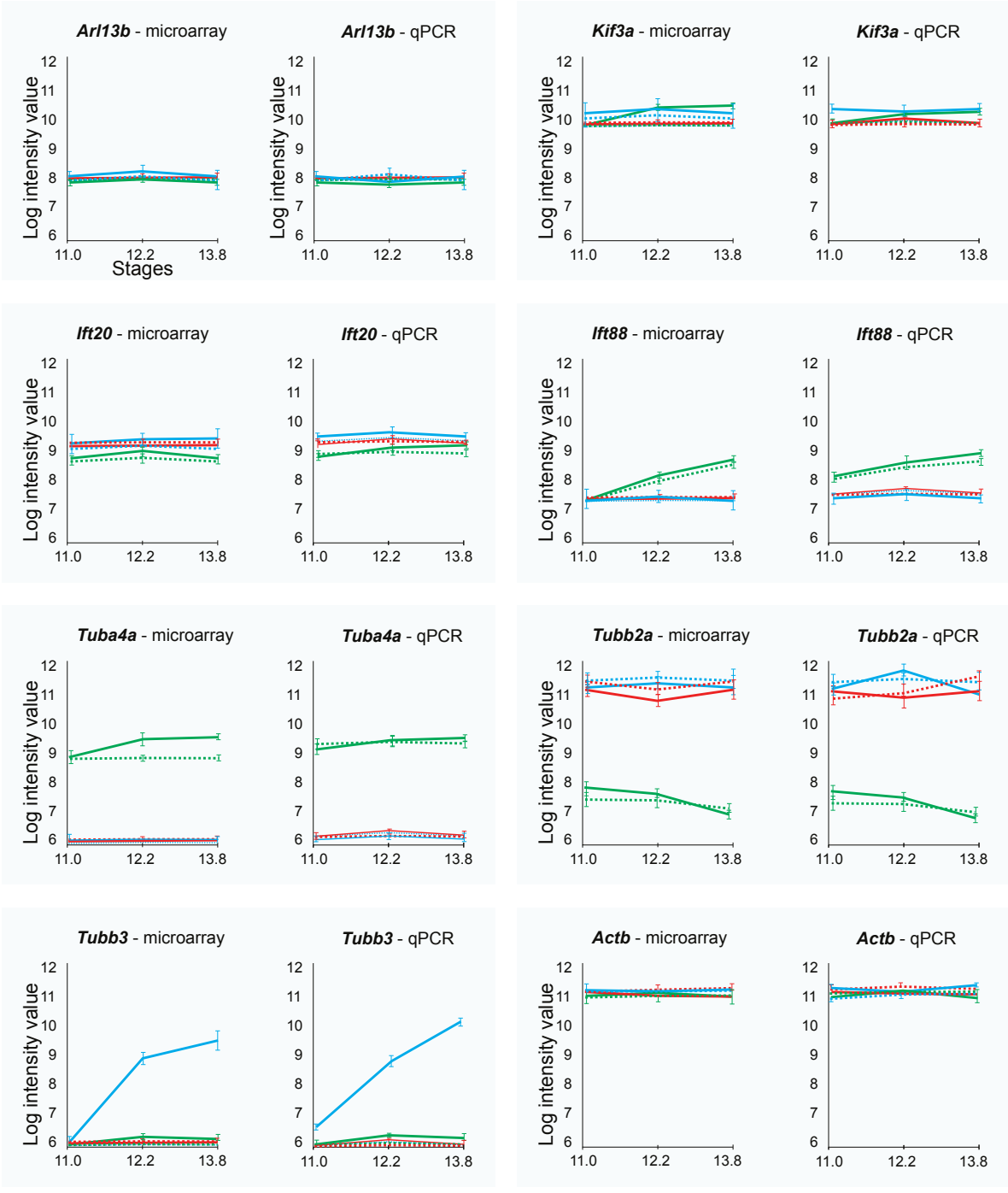

Fig. 1. Graphs of microarray validation. *Comparison of the log-normalized intensity values (6-12) of the expression of primary-cilia related genes* Arl13b, Kif3a, Ift20 *expressed ubiquitously at high level in three studied cell lines,* Ift88, Tuba4a *showing higher expression in the germ cells,* Tubb2a *showing lower expression in the germ cells,* Tubb3 *showing higher expression in XY supporting cells, and* Actb *as a positive control gene obtained via microarray supported by qPCR.*

primary cilia-related genes in different cell lines in differentiating gonads. Differences in the expression of primary cilia-related genes between somatic and germ cells suggest that, indeed, there is a difference in the function of primary cilia or primary cilia-related genes between somatic and germ cells. Wainwright and colleagues (2014) showed that mice with a mutation in *Ift144* (intraflagellar transport gene 144) gene had abnormally large gonads and more testis cords than control gonad (Wainwright *et al.*, 2014). This

TABLE 1

PRIMARY CILIA-RELATED GENES EXPRESSED UBIQUITOUSLY IN THE DEVELOPING MOUSE GONADS WITH NO SIGNIFICANT DIFFERENCES BETWEEN CELL TYPES

		Supporting cells		Interstitial/stromal cells		Germ cells	
Gene symbol	**Gene name**	**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	++	++	++	++	++	++
Cnp	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	+++	+++	+++	+++	+++	+++
Ift122	Intraflagellar transport protein 122 homolog	.	.	.	.	.	.

TABLE 1 (CONTINUED)

		Supporting cells		Interstitial/stromal cells		Germ cells	
Gene symbol	**Gene name**	**XX**	**XY**	**XX**	**XY**	**XX**	**XY**
Ift140	intraflagellar transport 140	+	+	+	+	+	+
Ift172	Intraflagellar transport protein 172 homolog	+	+	+	+	+	+
Ift20	Intraflagellar transport protein 20 homolog	++	++	++	++	++	++
Ift52	Intraflagellar transport protein 52 homolog	++	++	++	++	++	++
Ift57	Intraflagellar transport protein 57 homolog	++	++	++	++	++	++
Ift74	Intraflagellar transport protein 74 homolog	++	++	++	++	++	++
Ift80	Intraflagellar transport protein 80 homolog	++	++	++	++	++	++
Ift81	Intraflagellar transport protein 81 homolog	++	++	++	++	++	++
Ift88	Putative uncharacterized protein	+	+	+	+	+	+
Inpp5e	Inositol polyphosphate-5-phosphatase E	.	.	.	.	.	.
Invs	Inversin	+	+	+	+	+	+
Ipo5	Importin 5	++	++	++	++	++	++
Ipo7	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	.	.	.	.	.	.
Ppp2ca	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	+	+	+	+	+	+
Xpo7	Exportin 7	++	++	++	++	++	++
1500003O03	Uncharacterized protein	+	+	+	+	+	+

Primary cilia-related genes expressed ubiquitously in the 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)].

indicates that indeed this primary cilia-related gene is involved in gonad development. The transcriptome analysis presented in this study creates a valuable database, which will be crucial in further studies of the role of primary cilia or their related genes in the development and/or differentiation of the gonads.

Materials and Methods

The study had been approved by the 1st Local Commission for Ethics in Experiments on Animals. Five transgenic mouse lines were used to isolate the supporting, interstitial/stromal and germ cells as previously

TABLE 2

PRIMARY CILIA-RELATED GENES EXPRESSED AT HIGHER LEVEL IN GERM CELLS COMPARED TO GONADAL SOMATIC CELLS

		Supporting cells		Interstitial/stromal cells		Germ cells	
Gene symbol	**Gene name**	**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	.	.	.	.	++	++
Xpo5	Exportin 5	++	++	++	++	+++	+++

Primary cilia-related genes 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)].

TABLE 3

PRIMARY CILIA-RELATED GENES EXPRESSED AT LOWER LEVEL IN GERM CELLS COMPARED TO GONADAL SOMATIC CELLS

		Supporting cells		Interstitial/stromal cells		Germ cells	
Gene symbol	**Gene name**	**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)	++	++	++	++	+	+
Idh1	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	+++	+++	+++	+++	+	+

Primary cilia-related genes 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)].

TABLE 4

PRIMARY CILIA-RELATED GENES SHOWING SIGNIFICANT DIFFERENCES IN EXPRESSION LEVELS BETWEEN DIFFERENT CELL TYPE OR SEX

		Supporting cells		Interstitial/stromal cells		Germ cells	
Gene symbol	**Gene name**	**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	.	.	++	++	.	.

Primary cilia-related genes showing significant differences in the expression level (fold change ≥1.5) between different cell types or sex 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)].

described (Piprek *et al*., 2017). All individuals were genotyped to define sex and the presence of transgene as previously described (Piprek *et al*., 2017). The gonads from mouse fetuses were pooled accordingly to the sex and developmental stage. The gonads were incubated in 250 μl 0.25% Trypsin–EDTA (Sigma, #T4049) at 37°C for 5–10 minutes (Piprek *et al*., 2017). After tissue dissociation, the enzyme solution was replaced with PBS. Cells were centrifuged and the cell pellet was resuspended in PBS with Hoechst dye and incubated for 15 min. About 100,000 cells were isolated from one sample. Pooled gonads from 5 fetuses were used for each time point and experiments were repeated three times. Fluorescence-activated cell sorting (FACS) was used to segregate three cell types isolated from the gonads (Piprek *et al*., 2017). Total RNA was isolated from each cell type and analyzed using microarray technique as previously described (Piprek *et al*., 2017). Raw data were analyzed as previously described (Piprek *et al*., 2017), and normalized data are available in Gene Expression Omnibus (accession number GSE94806). Data obtained by microarray analysis were confirmed by real-time quantitative PCR (qPCR) of eight chosen genes. 50 ng RNA of each sample was reverse-transcribed into cDNA using random primers and SuperScript III Reverse Transcriptase (Invitrogen, 18080044). The qPCR procedure was performed in 5μl reactions using SYBR Green Master Mix (Life Technologies, 4312704) in the 7500 Fast Real-Time PCR System (Applied Biosystems) with universal cycling parameters and analyzed as previously described (Svingen *et al*., 2009; Piprek *et al*., 2017). Statistical analysis was performed using the nonparametric ANOVA Kruskal-Wallis test, Tukey's test and Statistica 7.0 software.

Acknowledgements

The study was conducted within the project financed by the National Science Centre assigned on the basis of the decision # DEC-2013/11/D/NZ3/00184.

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