Mammalian meiosis is more conserved by sex than by species: conserved co-expression networks of meiotic prophase

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Abstract

Despite the importance of meiosis to human reproduction, we know remarkably little about the genes and pathways that regulate meiotic progression through prophase in any mammalian species. Microarray expression profiles of mammalian gonads provide a valuable resource for probing gene networks. However, expression studies are confounded by mixed germ cell and somatic cell populations in the gonad and asynchronous germ cell populations. Further, widely used clustering methods for analyzing microarray profiles are unable to prioritize candidate genes for testing. To derive a comprehensive understanding of gene expression in mammalian meiotic prophase, we constructed conserved co-expression networks by linking expression profiles of male and female gonads across mouse and human. We demonstrate that conserved gene co-expression dramatically improved the accuracy of detecting known meiotic genes compared with using co-expression in individual studies. Interestingly, our results indicate that meiotic prophase is more conserved by sex than by species. The co-expression networks allowed us to identify genes involved in meiotic recombination, chromatin cohesion, and piRNA metabolism. Further, we were able to prioritize candidate genes based on quantitative co-expression links with known meiotic genes. Literature studies of these candidate genes suggest that some are human disease genes while others are associated with mammalian gonads. In conclusion, our co-expression networks provide a systematic understanding of cross-sex and cross-species conservations observed during meiotic prophase. This approach further allows us to prioritize candidate meiotic genes for in-depth mechanistic studies in the future.

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Introduction

Meiosis, the process by which haploid gametes are produced, is essential to human reproduction. The regulation of meiosis is highly complex and is further complicated by sexual dimorphism. Although meiotic phases are essentially the same in both sexes, fundamental differences exist in timing, outcome, and checkpoint control (Morelli & Cohen 2005, Bowles & Koopman 2007, Kimble & Page 2007, Hunt & Hassold 2008, Handel & Schimenti 2010). In the female, all oocytes enter meiosis semi-synchronously during a narrow window of fetal development. Meiosis arrests before birth at the end of prophase and resumes at puberty during each ovulation cycle. One egg is produced at the end of female meiosis. In contrast, meiosis in the male begins in the juvenile testis and is a continuous and unsynchronized process. Four sperms are generated from each male meiosis cycle. Any error during meiosis jeopardizes the quality and quantity of

gametes and can lead to infertility, birth defects, or miscarriages. Meiotic prophase is a particularly vulnerable time for errors because homologous chromosomes pair and exchange genetic information. Studies of mouse mutants for meiotic genes have revealed different levels of checkpoint control in males and females. Due to more stringent control mechanisms in the male that eliminate cells with errors, the probability of producing an aneuploid gamete is at least an order of magnitude greater in the female (Hunt & Hassold 2002, Morelli & Cohen 2005).

Despite the importance of meiosis to human reproduction, few genes are known to be critical to meiotic progression through prophase in mammals. Further, virtually no data exist to describe interactions between meiotic genes or functional pathways connecting them. Indeed, the overall structure of the functional and regulatory pathways directing germ cells into the meiotic program remains completely undefined. Identifying gene networks and pathways involved in meiosis is

necessary for bridging the knowledge gap to address health issues such as infertility and birth defects.

One resource that may allow the identification of gene networks is microarray, which provides a platform to evaluate genome-wide expression. Reproductive tissue-specific microarray profiles allow us to probe pathways and networks that may govern meiotic dynamics (Schultz *et al.* 2003, Shima *et al.* 2004, Small *et al.* 2005, Chalmel *et al.* 2007, Olesen *et al.* 2007, Houmard *et al.* 2009, Lawson *et al.* 2011, Rolland *et al.* 2011). However, the analysis has been limited to simple clustering of individual expression profiles, preventing construction of gene networks and prioritization of candidate genes for testing. Further, the meiotic process conserved between males and females has not been systematically investigated from these expression profiles.

Although studying meiosis in a single sex and species is important, cross-sex and cross-species investigation of meiotic pathways can help in identifying the most conserved players as well as in making inferences on human meiotic defects. In this study, our objective was to derive a comprehensive understanding of meiotic prophase genes conserved across species and sex. To this end, we constructed conserved gene co-expression networks by integrating microarray profiles. We focused on time-course expression profiles covering meiotic prophase during fetal oogenesis and the first wave of spermatogenesis in both human and mouse (Shima *et al.* 2004, Small *et al.* 2005, Houmard *et al.* 2009), since the mouse serves as a genetically tractable mammalian model for investigating human meiosis. Prophase is the most synchronized meiotic process, providing a favorable temporal window for investigating genetic control of meiosis in both sexes. Further, prophase is the most defining stage of meiosis, during which homologous chromosomes pair and undergo recombination. Our conserved co-expression networks provide a systematic understanding of potential interactions between genes in prophase. Cross-species and cross-sex inference allowed us to identify core components of meiotic machinery and to prioritize candidate genes for experimental studies in mice and humans.

Results

Conserved co-expression networks of mammalian meiotic prophase

To construct conserved meiotic pathways in mammals, we took advantage of available prophase expression profiles from males and females across human and mouse (Shima *et al.* 2004, Small *et al.* 2005, Houmard *et al.* 2009). We identified 16 376 genes conserved between human and mouse (called MHs) and investigated meiotic expression of these MHs (Table 1; Ostlund *et al.* 2010). Co-expression of MH gene pairs in prophase

Table 1 MHs are genes conserved between mouse and human.

	Gene number	Genome coverage ^a (%)	Genes per MH
MH	16 376	–	–
Mouse	16 566	71	1.01
Human	16 700	77	1.02

^aThe genome coverage was calculated based on total protein numbers in the mouse (23 497) and the human (21 673) from InParanoid Version 7.0 (Ostlund *et al.* 2010).

was first quantified from three individual microarray studies on the mouse postnatal testis, mouse embryonic ovary, and human fetal ovary using the Pearson correlation and a resulting standard z-score. Microarray data are currently unavailable for the human male. Then, we averaged z-scores across sex and species to determine conserved co-expression. We have developed an efficient ‘divide and conquer’ algorithm to process hundreds of millions of gene pairs using a standard desktop computer. MH gene pairs with average z-scores greater than a threshold can be connected to form a network. In this way, we constructed three co-expression networks of mammalian meiotic prophase: a network conserved between male mouse and female mouse (M_m-M_f), a network conserved between female mouse and female human (M_f-H_f), and a network conserved among male mouse, female mouse, and female human ($M_m-M_f-H_f$; Fig. 1).

Figure 1 Construction of conserved co-expression networks of mammalian meiotic prophase. Co-expression of two genes was calculated from each of three time-course microarray studies on male mouse (M_m), female mouse (M_f), and female human (H_f) using a standard z-score. For instance, the standard z-score for genes A and B is equal to 5 in the male mouse study. To identify conserved co-expression across species and sex, we averaged standard z-scores from different microarray studies for the same gene pair. For instance, the average z-score for genes A and B between male mouse and female mouse equals 4 by taking the average of 5 and 3. The top two gene pairs with greater z-scores are connected to form a network. This leads to the construction of three networks of mammalian meiotic prophase: a co-expression network conserved between male mouse and female mouse (M_m-M_f), a co-expression network conserved between female mouse and female human (M_f-H_f), and a co-expression network conserved among male mouse, female mouse, and female human ($M_m-M_f-H_f$).

Validation of conserved co-expression networks

We used known meiotic genes annotated by Gene Ontology (GO:0007126) as the standard to evaluate conserved co-expression networks. We created the precision–coverage curve, the appropriate metric to determine the performance of co-expression networks in identifying known meiotic genes (Fig. 2). All gene pairs in each network were sorted in descending order of average z-score, and precision and coverage were calculated in 200-pair increments. Precision is the ratio of known meiotic genes to all genes among co-expression pairs, while coverage is the number of known meiotic genes (the numerator of precision). Perfect precision (= 1) means every gene among co-expression pairs is a meiotic gene. Perfect coverage means genes among co-expression pairs cover all known meiotic genes. It is observed that low precision (y-axis) is due to the small number of known meiotic genes (x-axis) compared with the total number of genes genome-wide. There are ~80 meiotic genes among 16 376 MHs. Thus, the precision of identifying meiotic genes at random is ~0.005.

When we used mouse meiotic genes to evaluate conserved co-expression, we found that top pairs in all three networks can recover significant numbers of known meiotic genes compared with the randomized networks (Fig. 2A). In particular, the top 200 gene pairs (the first data point from the left) in the M_m – M_f – H_f network exhibit over an eightfold increase in precision. This suggests that multiple species integration can

improve the accuracy of meiotic gene identification. The top 200 gene pairs in M_m – M_f and M_f – H_f show a three- and five-fold increase in precision, respectively, compared with the random. Interestingly, the top 200 pairs in the M_f – H_f network contain a higher fraction of meiotic genes than those in M_m – M_f , indicating that meiosis is more conserved by sex than by species. When we used human meiotic genes to evaluate conserved co-expression in two networks that contain human genes, we found similar patterns: top pairs in co-expression networks were more enriched for known meiotic genes than the randomized networks, and the M_m – M_f – H_f network exhibited higher precision in identifying known meiotic genes than the M_f – H_f network (Fig. 2B).

To determine whether conserved co-expression across species and sex can improve the identification of known meiotic genes, we created precision–coverage curves for co-expression pairs from individual studies (Fig. 2C and D). These co-expression pairs were sorted in descending order of standard z-score, and curves were created in 200-pair increments. Top pairs from both female mouse and female human studies failed to identify known meiotic genes. Only the top co-expression pairs from the male mouse study achieved similar precision of meiotic gene identification as those in the M_m – M_f network. In meiotic gene, coverage of 13, M_m – M_f – H_f achieved 4.1% precision in identifying mouse meiotic genes while precisions of 1.2 and 0.6% were reached for networks of male mouse and female mouse respectively (Fig. 2A and C). In meiotic gene, coverage of 8, M_f – H_f

Figure 2 Co-expression across species and sex improves the identification of known meiotic genes compared with co-expression in individual studies. (A) Evaluation of conserved co-expression networks using known mouse meiotic genes. Conserved co-expression pairs were sorted in descending order of average z-score. To create the observed curve, precision and the number of meiotic genes were calculated from sorted pairs with an increment of 200 pairs. Precision is the fraction of meiotic genes to all genes among gene pairs. Random curves were derived from the average of 100 simulations of randomized gene pairs. (B) Evaluation of conserved co-expression networks using known human meiotic genes. The curves were similarly created as described in A. (C) Evaluation of co-expression in individual mouse studies using known mouse meiotic genes. Co-expression pairs were sorted in descending order of standard z-score. To create the observed curve, precision and the number of meiotic genes were calculated from sorted pairs with an increment of 200 pairs. Random curves were derived from the average of 100 simulations of randomized gene pairs. (D) Evaluation of co-expression in the human study using known human meiotic genes. The curves were similarly created as described in C.

achieved 2.5% precision in identifying mouse meiotic genes while precision of 0.5% was reached for the female mouse network (Fig. 2A and C). The precision of M_m-M_f was 1.6% in meiotic gene coverage of 6, similar to that of male mouse study but higher than the 0.5% precision of female mouse study (Fig. 2A and C). The comparison of precision using human meiotic genes reached the similar results as those using mouse meiotic genes (Fig. 2B and D). Our goal is to develop a computational method to efficiently predict novel meiotic genes, not to identify all meiotic genes. The prediction accuracy of top-ranked gene pairs is much more interesting than the overall accuracy. Therefore, comparison of precision at a small coverage is most relevant to this goal.

The comparison between co-expression across species vs co-expression in individual species strongly supported the notion that cross-species integration of expression profiles can significantly improve the accuracy of identifying known meiotic genes and thus the accuracy of predicting novel meiotic genes. Further, cross-species integration can help us understand the meiotic differences between sex and species. We demonstrated that these results are not affected by the number of sampling gene pairs or by the use of ranked genes (Supplementary Figures 1 and 2, see section on supplementary data given at the end of this article).

Because the top 200 gene pairs in each co-expression network showed highest precision of meiotic gene identification (Fig. 2A and B), we calculated significantly enriched GO terms among mouse (Table 2) and human genes (Table 3) from them. Consistent with the precision-coverage results, we found that more meiosis-related GO terms were associated with the $M_m-M_f-H_f$ network than with the other two networks. Enriched meiosis GO terms were similar when using mouse genes and human genes. We observed that terms describing the general meiotic process were enriched, including 'meiosis', 'male meiosis', and 'spermatogenesis'. GO terms associated with prophase were also enriched, such as 'meiotic prophase I', 'male meiosis I', 'pachytene', 'synaptonemal complex assembly', 'synapsis', 'reciprocal meiotic recombination', and 'female meiosis sister chromatid cohesion'. Finally, 'piRNA metabolic process' was a significantly enriched GO term in networks M_m-M_f and $M_m-M_f-H_f$, which contain male expression profiles. This is consistent with the evidence that the piRNA regulatory pathway influences DNA methylation of retrotransposons in the male germ cell (Aravin *et al.* 2007).

We observed that the top 200 co-expression pairs in the M_f-H_f network recover a higher fraction of known meiotic genes than those of M_m-M_f (Fig. 2A). We investigated whether this observation is related to absolute expression levels. We identified the maximum signal intensity for each gene across prophase in each study. Then, we classified each gene as either 'highly expressed' or 'lowly expressed' based on whether the

maximum signal intensity of the gene was higher or lower than the genome-wide median value. In this way, we grouped the top 200 gene pairs from the M_m-M_f network into one of the three categories: both genes were highly expressed (high, high), both were lowly expressed (low, low), or one was lowly expressed while

Table 2 Significant GO terms enriched among mouse genes from the top 200 MH gene pairs in co-expression networks^a.

GO term	GO term name ^b	Hypergeometric <i>P</i> value ^c
M_m-M_f		
GO:0006596	Polyamine biosynthetic process	0.000
GO:0009267	Cellular response to starvation	0.001
GO:0007126	Meiosis	0.002
GO:0007283	Spermatogenesis	0.002
GO:0034587	piRNA metabolic process	0.002
GO:0007182	Common partner SMAD protein phosphorylation	0.002
GO:0002309	T cell proliferation during immune response	0.002
GO:0050435	β -Amyloid metabolic process	0.002
GO:0006597	Spermine biosynthetic process	0.002
GO:0044267	Cellular protein metabolic process	0.002
GO:0031047	Gene silencing by RNA	0.003
GO:0051101	Regulation of DNA binding	0.005
GO:0008295	Spermidine biosynthetic process	0.005
GO:0006857	Oligopeptide transport	0.008
M_f-H_f		
GO:0019478	D-amino acid catabolic process	0.001
GO:0007140	Male meiosis	0.002
GO:0001657	Ureteric bud development	0.002
GO:0007286	Spermatid development	0.003
GO:0019216	Regulation of lipid metabolic process	0.003
GO:0000239	Pachytene	0.005
GO:0009888	Tissue development	0.005
GO:0051056	Regulation of small GTPase-mediated signal transduction	0.005
GO:0030199	Collagen fibril organization	0.006
GO:0007156	Homophilic cell adhesion	0.007
GO:0007131	Reciprocal meiotic recombination	0.008
GO:0007141	Male meiosis I	0.008
GO:0019229	Regulation of vasoconstriction	0.008
$M_m-M_f-H_f$		
GO:0007126	Meiosis	0.000
GO:0007129	Synapsis	0.000
GO:0007130	Synaptonemal complex assembly	0.000
GO:0007140	Male meiosis	0.000
GO:0007286	Spermatid development	0.000
GO:0060395	SMAD protein signal transduction	0.000
GO:0007283	Spermatogenesis	0.001
GO:0060433	Bronchus development	0.001
GO:0009566	Fertilization	0.001
GO:0060425	Lung morphogenesis	0.001
GO:0007066	Female meiosis sister chromatid cohesion	0.003
GO:0034587	piRNA metabolic process	0.003
GO:0003014	Renal system process	0.003
GO:0000239	Pachytene	0.005
GO:0007128	Meiotic prophase I	0.005
GO:0030199	Collagen fibril organization	0.006
GO:0043046	DNA methylation during gametogenesis	0.008
GO:0007131	Reciprocal meiotic recombination	0.008
GO:0007141	Male meiosis I	0.008

^aThe top MH gene pairs are the pairs with the highest average z-scores in co-expression networks. ^bGO names related to meiosis are in bold. ^cGO term significance was defined by hypergeometric *P* value < 0.01.

Table 3 Significant GO terms enriched among human genes from the top 200 MH gene pairs in co-expression networks^a.

GO term	GO term name ^b	Hypergeometric P value ^c
M_f-H_f		
GO:0007130	Synaptonemal complex assembly	0.000
GO:0008217	Regulation of blood pressure	0.001
GO:0001657	Ureteric bud development	0.001
GO:0019478	D-amino acid catabolic process	0.001
GO:0007286	Spermatid development	0.003
GO:0009566	Fertilization	0.003
GO:0010459	Negative regulation of heart rate	0.003
GO:0000239	Pachytene	0.005
GO:0035023	Regulation of Rho protein signal transduction	0.005
GO:0031100	Organ regeneration	0.005
GO:0030240	Muscle thin filament assembly	0.005
GO:0007320	Insemination	0.005
GO:0042475	Odontogenesis of dentine-containing tooth	0.006
GO:0007155	Cell adhesion	0.006
GO:0007128	Meiotic prophase I	0.008
GO:0043547	Positive regulation of GTPase activity	0.008
$M_m-M_f-H_f$		
GO:0007126	Meiosis	0.000
GO:0007130	Synaptonemal complex assembly	0.000
GO:0007128	Meiotic prophase I	0.000
GO:0007286	Spermatid development	0.000
GO:0009566	Fertilization	0.000
GO:0007141	Male meiosis I	0.001
GO:0030308	Negative regulation of cell growth	0.002
GO:0007283	Spermatogenesis	0.003
GO:0007140	Male meiosis	0.003
GO:0008156	Negative regulation of DNA replication	0.004
GO:0007129	Synapsis	0.005
GO:0000239	Pachytene	0.005
GO:0045217	Cell-cell junction maintenance	0.005
GO:0030240	Muscle thin filament assembly	0.005
GO:0048268	Clathrin coat assembly	0.005
GO:0043587	Tongue morphogenesis	0.005
GO:0034587	piRNA metabolic process	0.008
GO:0001539	Ciliary or flagellar motility	0.008
GO:0007131	Reciprocal meiotic recombination	0.009

^aThe top MH gene pairs are the pairs with the highest average z-scores in co-expression networks. ^bGO names related to meiosis are in bold.

^cGO term significance was defined by hypergeometric P value <0.01.

the other was highly expressed (low, high). This grouping was performed for the male mouse study and the female mouse study respectively. We found that the expression level of female mouse genes was similar to that of male mouse genes, with 70% (33.5%+26.5%+9.5%) of pairs in the same expression category (Fig. 3A). On the other hand, when we grouped the top 200 gene pairs from the M_f-H_f network into three categories for the female mouse study and the female human study, respectively, only 52% (23.0%+21.0%+8.0%) of pairs showed consistent expression (Fig. 3B). This observation indicates that similar meiotic profile between female mice and female humans seen in Fig. 2A is not due to absolute expression levels. To compare genome-wide expression profile, we obtained

the maximum signal intensity for each gene across prophase in each study and subsequently calculated Pearson correlation between three studies. We found that the expression profile of female mice is more similar to male mice (Pearson coefficient=0.89) than female humans (Pearson coefficient=0.41). This indicates that global expression similarity also does not contribute to the result that top pairs in the M_f-H_f network recovers a higher fraction of known meiotic genes than those of M_m-M_f . The most likely explanation is that more meiotic genes share similar expression trend over the time course of prophase between female mouse and female human than between female mouse and male mouse.

An alternative approach to identify conserved co-expression

An alternative to quantify conserved co-expression is to use the minimum standard z-score across species and sex (Langfelder & Horvath 2007). We found that our conclusions drawn from the precision-coverage curves with the average z-score still hold true when the minimum z-score is used: 1) top co-expression pairs in the observed networks are more enriched for known meiotic genes than those in the randomized networks, 2) the M_f-H_f network contains a higher fraction of meiotic genes than M_m-M_f and 3) top co-expression pairs in the conserved networks exhibited higher precision in identifying known meiotic genes than those in individual species networks (compare Supplementary Figure 3 (see section on supplementary data given at the end of this article) with Fig. 2C and D). Compared with the use of average z-score, the use of the minimum z-score improved the performance of M_m-M_f and M_f-H_f in identifying known meiotic genes but decreased the performance of $M_m-M_f-H_f$ (compare Supplementary Figure 3 with Fig. 2A and B). This is because the minimum is more vulnerable to outliers than the average. The use of the average z-score to identify conserved co-expression becomes more robust when the number of integrated species is increased.

Characterization of conserved co-expression networks

We quantified the functional association of conserved co-expression pairs using semantic similarity in the GO sub-ontology Biological Process (Lord *et al.* 2003; Fig. 4). We observed that the top co-expression pairs in all three networks exhibited closer functional association than those in randomized networks. The semantic similarity gradually declined when we proceeded the sorted list of co-expression pairs. In particular, the top co-expression pairs in $M_m-M_f-H_f$ showed tighter functional connections than those in the other two networks. This indicates that multiple species integration

Figure 3 Expression level of the top 200 MH gene pairs from conserved co-expression networks. Gene pairs were classified as (high, high) if expression levels of both genes were higher than the median of genome-wide levels. Gene pairs were classified as (low, low) if expression levels of both genes were lower than the median of genome-wide levels. Gene pairs were classified as (low, high) if the expression level of one gene was lower than the median of genome-wide levels while the other was higher. (A) The M_m - M_f network. (B) The M_f - H_f network.

can recover reliable predictions on gene functional associations. Results from the human GO agree with results from the mouse GO.

To examine whether top co-expression pairs exhibit physical interactions, we retrieved 23 378 mouse protein-protein interactions and 227 789 human protein-protein interactions from various databases (Bader *et al.* 2003, Salwinski *et al.* 2004, Stark *et al.* 2006, Chatr-aryamontri *et al.* 2007, Kerrien *et al.* 2007, Keshava Prasad *et al.* 2009). However, we observed that very few top co-expression pairs from any network overlapped with known human or mouse protein interactions. We believe that the low coverage of protein-protein interactions in mammals contributes to this result. Indeed, only one out of 6000 mouse gene pairs and one out of 600 human gene pairs would show physical interactions by chance according to the current coverage.

We tested whether genes in the co-expression networks are enriched for essential genes, i.e. those required for viability. Two genome-wide RNAi knock-down screens identified 461 human essential genes with conservation in the mouse (Schlabach *et al.* 2008, Silva *et al.* 2008). Mouse essential genes were compiled from the database of Mouse Genome Informatics (Liao & Zhang 2008), and 1647 are conserved in the human. We found that the fraction of essential genes was slightly higher in the observed networks than in the randomized

networks for both mouse and human (Fig. 5). The percentage of mouse essential genes slowly declines along the sorted list of co-expression pairs, suggesting that top co-expression pairs are more enriched with mouse essential genes (Fig. 5A). In particular, the M_m - M_f - H_f network was most enriched with mouse essential genes. This indicates that multiple species integration recovers the most conserved and essential part of cellular machinery.

Biological networks are scale free, which means that few nodes (hubs) in the network have a large number of directly connecting neighbors and a large number of nodes have few neighbors (Barabasi & Oltvai 2004). Thus, under a scale-free architecture, the degree distribution decreases exponentially with the degree, where degree is the number of direct neighbors for a given node. We found that all three conserved co-expression networks displayed scale-free properties, and genes in M_m - M_f - H_f and M_f - H_f were more connected than those in M_m - M_f (Fig. 6A). This inhomogeneous connectivity indicates that a small number of genes show co-expression with many other genes while a large number of genes exhibit co-expression with very few genes. Such architecture makes the network robust against random removal of a large fraction of genes. The clustering coefficient measures how well the neighbors of a node are connected. A value of 1 indicates that all neighbors of the node are

Figure 4 GO semantic similarity of top MH gene pairs in conserved co-expression networks. Semantic similarity was calculated using GO sub-ontology Biological Process. The randomized curves were derived from the average of 100 simulations of randomized gene pairs. (A) Mouse GO semantic similarity. (B) Human GO semantic similarity.

Figure 5 The enrichment of essential genes among top MH gene pairs from conserved co-expression networks. The randomized curves were derived from the average of 100 simulations of randomized gene pairs. (A) Mouse essential genes. (B) Human essential genes.

connected with each other; a value of 0 indicates no connections in the neighborhood. The distribution of clustering coefficients displayed similar patterns for all three networks (Fig. 6B). The majority of genes had coefficients of 0; ~10% of genes had fully connected neighborhood. Overall, the global network properties are highly comparable among three conserved co-expression networks.

Prediction of novel meiotic genes from conserved co-expression networks

Conserved co-expression networks identified genes displaying similar expression across species and sex during meiotic prophase. Hence, a co-expression network can serve as a topological map to indicate potential functional connections among genes. To predict novel meiotic genes based on conserved co-expression, we focused on the top 200 pairs in three networks, M_m-M_f , M_f-H_f and $M_m-M_f-H_f$. The rationale is that they are most enriched with known meiotic genes (Fig. 2A and B) and exhibit the closest functional associations (Fig. 4). The top 200 gene pairs for each network are listed in [Supplementary Table 1](#) (see section on [supplementary data](#) given at the end of this article) while the gene pairs that appeared in more than one network are summarized in [Table 4](#).

Gene clusters containing known mouse or human meiotic genes were isolated from the networks for display (Fig. 7). We used mouse gene names to label MH genes because mouse is present in every network. Four functional categories of known meiotic genes were identified from the networks: components of the synaptonemal complex (*Sycp2*, *Sycp3*, *Tex11*, and *Tex15*), meiosis-specific cohesins (*Smc1b* and *Stag3*), meiotic recombination machinery (*Dmc1*, *Msh4*, and *Trip13*), and genes involved in the piRNA pathway (*Piwi2*, *Tdrd1*, and *Mael*). We focused on candidate meiotic genes that exhibited direct co-expression links with known meiotic genes. Some of these candidates are supported by published experiments for their functions in testis and ovary development, yet others are entirely new with no previous associations with meiosis. For example, many human disease genes, including tumor-suppressor genes, displayed direct co-expression links with known meiotic genes.

The synaptonemal complex consists of two lateral elements and the transverse filament in the middle. This structure results from the pairing of two homologous chromosomes and allows for recombination between the homologous chromosomes. SYCP2 and SYCP3 are components of the lateral elements. The co-expression link between *Sycp2* and *Tex11* in the M_f-H_f network is well supported by the observed physical interaction between the encoded proteins (Yang et al. 2008b). *Sycp2*

Figure 6 Network properties of conserved co-expressions networks. Results were derived from the top 5000 MH gene pairs; results were similar by including more or fewer numbers of gene pairs. (A) The degree distribution. Degree is the number of direct neighbors a gene has. (B) The distribution of clustering coefficients. Clustering coefficient measures the neighborhood connectivity around a gene.

Table 4 The top 200 MH gene pairs common to at least two out of three co-expression networks^a.

MH pair using mouse gene symbols	M_m-M_f	M_f-H_f	$M_m-M_f-H_f$
<i>Lpp-Postn</i>	+	+	+
<i>Rg9mtd2-Zfp238</i>	+	+	+
<i>Trim23-Zhx1</i>	+	+	+
<i>Ikbkap-Nans</i>	+	+	+
<i>Anxa9-Sipa1l2</i>	+	+	+
<i>Ncam2-Ndufs5</i>	+	+	+
<i>Dnaja4-Serinc3</i>	+	+	+
<i>Adams2-Col6a3</i>	+	+	+
<i>Ccdc74a-Fbxl8</i>	+	+	+
<i>Birc2-Mrpl11</i>	+	+	+
<i>A630055G03Rik-Zc3h12c</i>	+	+	+
<i>Rab21-Scly</i>	+		+
<i>1810074P20Rik-Mtmr6</i>	+		+
<i>Dmc1-Smc1b</i>	+		+
<i>Clock-Rabgap1l</i>	+		+
<i>1700021F05Rik-Hyal1</i>	+		+
<i>6030405A18Rik-Pard3</i>	+		+
<i>Nlgn2-Rsad2</i>	+		+
<i>Agtr2-Fbxl7</i>	+		+
<i>Ghitm-Hoxc13</i>	+		+
<i>Dyx1c1-Lrrc50</i>	+		+
<i>Pml-Rgs11</i>	+		+
<i>Cgn-Eef2</i>	+		+
<i>Pign-Prei4</i>	+		+
<i>Armcx6-Lox</i>	+		+
<i>4930473A06Rik-Smc1b</i>	+		+
<i>Stk22s1-Wnk4</i>	+		+
<i>Alg8-Car5a</i>	+		+
<i>Cnot6-Smad6</i>	+		+
<i>Kdsr-Xrn1</i>	+		+
<i>Eif2ak4-Pcdh22</i>	+		+
<i>4932438A13Rik-Trim23</i>	+		+
<i>Dnahc8-Tcte3</i>	+		+
<i>Pogz-Rcbtb1</i>	+		+
<i>1600027N09Rik-Pex7</i>	+		+
<i>Dnahc8-Lypd4</i>	+		+
<i>Stk33-Zpbb</i>	+		+
<i>Fbxo36-Sccpdh</i>	+		+
<i>Apba3-Fut11</i>	+		+
<i>Eif3eip-Nans</i>	+		+
<i>Glyat-Ren2</i>		+	+
<i>4921507P07Rik-Rad17</i>		+	+
<i>Ccdc93-Phlpp</i>		+	+
<i>Spaca1-Tex12</i>		+	+
<i>Cd248-Cypr1</i>		+	+
<i>1700123I01Rik-Smc1b</i>		+	+
<i>Oip5-Pik3r3</i>		+	+
<i>Dmap1-Dnase1</i>		+	+
<i>Adad1-Hormad2</i>		+	+
<i>Afp-Cldn2</i>		+	+
<i>Rps6ka3-Trip13</i>		+	+
<i>Piwil2-Zfp473</i>		+	+
<i>Lrrc51-Slc25a19</i>		+	+
<i>Smtnl2-Zfp467</i>		+	+
<i>Glis2-Rgs3</i>		+	+
<i>Fcgrt-Mpz</i>		+	+
<i>Bbs4-Steap1</i>		+	+
<i>Eif1b-Tmem135</i>		+	+
<i>Stc1-Trps1</i>		+	+
<i>Dgat2-Rab28</i>		+	+
<i>Ints6-Smc1b</i>		+	+
<i>Dmrtc2-Hormad2</i>		+	+
<i>Bmp4-Tcf21</i>		+	+
<i>Ppp2r1a-Spr</i>		+	+
<i>Gjb6-Pck1</i>		+	+
<i>1700123I01Rik-Ints6</i>		+	+

Table 4 Continued.

MH pair using mouse gene symbols	M_m-M_f	M_f-H_f	$M_m-M_f-H_f$
<i>Chl1-Rgl1</i>		+	+
<i>Ccdc21-Sepsecs</i>		+	+
<i>Gabarapl2-Lrrfip2</i>		+	+
<i>Ogn-Sdpr</i>		+	+
<i>Garnl1-Taf12</i>		+	+
<i>0610010D20Rik-Bbox1</i>		+	+
<i>Acta1-Actc1</i>		+	+
<i>Tex15-Ugt8a</i>		+	+
<i>Acta1-Mylpf</i>		+	+
<i>Armcx3-Rbms1</i>		+	+
<i>Smtnl2-Tgfb1i1</i>		+	+
<i>Dmc1-Ints6</i>		+	+
<i>Nrgn-Setd1a</i>		+	+
<i>Myl1-Vgll2</i>		+	+
<i>2310065K24Rik-Dnase1</i>		+	+
<i>4931406P16Rik-Mfsd11</i>		+	+
<i>1700123I01Rik-Dmc1</i>		+	+
<i>Acta1-Casq2</i>		+	+

^aThe top MH gene pairs are the pairs with the highest average z-scores in co-expression networks.

is connected with several non-meiotic genes in addition to *Sycp3* in the $M_m-M_f-H_f$ network, including *Hspa4l*, *Hsf2bp*, *Trf*, and *Hormad2*. *Tex11* and *Tex15* are required for chromosomal synapsis and recombination (Yang *et al.* 2008a, 2008b). *Tex11* is connected to *Hspa4l* in M_f-H_f and to *Krit1* in $M_m-M_f-H_f$, while *Tex15* is connected to *Ugt8a* in both networks. Among the candidate meiotic genes, *Hspa4l* and *Hsf2bp* are heat-shock proteins predominantly expressed in the testis (Yoshima *et al.* 1998, Held *et al.* 2006). In particular, *Hspa4l*-deficient mice display reduced male fertility. These findings are consistent with our prediction that *Hspa4l* and *Hsf2bp* might participate in synapsis and recombination. *Trf* also exhibits expression in the testis, and its protein level correlates with sperm yield in several mammalian species (Lecureuil *et al.* 2007). *Hormad2* was shown to associate with unsynapsed chromosome axes during meiotic prophase (Wojtasz *et al.* 2009), which supports our prediction for the role of *Hormad2* in the formation of the synaptonemal complex. Finally, *Krit1*, containing four ankyrin repeats, is expressed in the testis, and mutations in the gene result in cerebral cavernous malformations (Kehrer-Sawatzki *et al.* 2002).

Meiosis-specific cohesion proteins are closely aligned with SYCP2 and SYCP3 to form the lateral elements. They play a key role in synapsis, recombination, and chromosome segregation. We observed that cohesin *Smc1b* has a co-expression link with *Dmc1* in M_m-M_f and $M_m-M_f-H_f$, suggesting concordant expression of sister chromatid cohesion and early homologous recombination events during meiotic prophase. *Smc1b* is also connected with non-meiotic genes *Ints6* and *Krit1* in M_f-H_f and *Ints6* and *Atf7ip2* in $M_m-M_f-H_f$. Particularly, *Smc1b*, *Dmc1*, *Ints6*, and *Atf7ip2* form a four-node

Figure 7 The conserved co-expression networks. Connecting the top 200 MH gene pairs formed the networks. Clusters containing known mouse or human meiotic genes were displayed in the figure. Genes were labeled with mouse gene symbols. (A) The M_m - M_f network. (B) The M_f - H_f network. (C) The M_m - M_f - H_f network.

clique (each node has a connection to each of the others) in M_m - M_f - H_f , suggesting related functions of these genes. Additionally, a couple of putative uncharacterized genes are directly linked to *Smc1b*. In contrast to the high connectivity of *Smc1b*, *Stag3* only connects to the non-meiotic gene *Crebl2* in M_m - M_f - H_f . Among the candidate meiotic genes that are linked to cohesins, *Ints6* and *Crebl2* are tumor suppressor genes and participate in cell cycle regulation (Thomson *et al.* 2008, Filleur *et al.* 2009). *Atf7ip2* is expressed both in the embryonic gonad and continuously in the testis from newborn to adult, suggesting a potential role in transcription modulation during gonad development (Cai *et al.* 2006).

Meiotic recombination occurs in the context of the synaptonemal complex. The process is initiated by double-strand breaks, followed by strand invasion, and the formation of double Holliday junctions. Resolution of Holliday junctions results in chromosomal crossovers. *Dmc1*, which encodes a strand-invasion protein, connects with cohesin *Smc1b* in all three networks. MSH4 is a mismatch repair protein, and *Msh4* links to *Hsp41* and *Mtl5* in the M_m - M_f - H_f network. *Mtl5* encodes a metallothionein-like protein, which is expressed in both male and female meiotic prophase (Olesen *et al.* 2004). *Trip13* is required for meiotic recombination (Li & Schimenti 2007, Roig *et al.* 2010) and links with *Rps6ka3* in both M_f - H_f and M_m - M_f - H_f . The role of *Rps6ka3* in meiosis has never been described, but mutations in this gene, which encodes a growth factor-regulated serine-threonine protein kinase, have been associated with Coffin-Lowry syndrome, an X-linked disorder characterized by retardation and skeletal deformations (Delaunoy *et al.* 2001).

Interestingly, several genes involved in piRNA metabolism were identified from the conserved co-expression networks. Three Miwi genes, *Piwi1*, *Piwi2*, and *Piwi4*,

are mainly expressed in the male germ cell; each is required for spermatogenesis (Deng & Lin 2002, Kuramochi-Miyagawa *et al.* 2004, Carmell *et al.* 2007). The Miwi-piRNA complex causes retrotransposon silencing via DNA methylation and thus prevents DNA damage in the germ cell. We found that *Zfp473* is linked to *Piwi2* in both M_f - H_f and M_m - M_f - H_f . *Piwi2* is the only Miwi expressed in the prenatal oocyte (Watanabe *et al.* 2008). This is consistent with the appearance of *Piwi2* in the female-containing networks. The link between *Piwi2* and *Zfp473* suggests that *Zfp473* might function as a germ cell-specific transcription factor during meiosis. MAEL forms a complex with piRNAs and Miwi proteins and plays a central role during spermatogenesis by repressing transposable elements (Soper *et al.* 2008). *Mael* connects with *Cdan1* and *Ttbk2* in M_m - M_f and with *Stk31* in M_m - M_f - H_f . Mutations in *Cdan1* cause congenital dyserythropoietic anemia type I, while mutations in *Ttbk2* lead to the neurodegenerative disease spinocerebellar ataxia type 11 (Dgany *et al.* 2002, Houlden *et al.* 2007). STK31 is a serine-threonine kinase, which displays testis-specific expression as well as high expression in colorectal cancer samples (Yokoe *et al.* 2008). Meiotic functions of these three genes have never been described earlier.

Discussion

Meiosis is an essential developmental program that determines the quality and quantity of the next generation. Any error during this process can lead to infertility, miscarriages, or birth defects. Recently, considerable interest has focused on the derivation of functional gametes from embryonic stem cells or induced pluripotent stem cells *in vitro* (Geijsen *et al.* 2004, Nayernia *et al.* 2006). However, the development of such technologies requires a thorough

understanding of a highly complex series of inter-related pathways leading to germ cell entry and progress through meiosis. Presently, such information is lacking: only a very limited number of meiotic genes have been characterized, and even less is known about the interactions between meiotic genes. A better understanding of the meiotic program will improve our ability to generate therapeutic strategies to overcome meiotic defects.

Microarray profiles of testis and ovary simultaneously capture expression of thousands of genes during meiosis. However, gonad samples contain mixed germ cells and somatic cells, and not all germ cells undergo meiosis in a synchronized fashion. Further, due to the difficulties of retrieving gonads, only limited time points have been covered in expression studies. Therefore, the quality of expression profiles of mammalian gonads is inferior to that of model organisms such as yeast. Under such circumstances, bioinformatics approaches become necessary to remove noise and decode germ cell signals from gonad microarray profiles.

We used the Pearson correlation and a resulting z-score to identify conserved gene pairs displaying co-expression in male and female gonads across mouse and human. Further, we developed an efficient 'divide and conquer' algorithm to implement our method on a standard desktop computer. Our conserved co-expression networks provide a comprehensive understanding of real and potential interactions between genes in mammalian meiotic prophase. This approach significantly improved the identification of known meiotic genes compared with examining individual expression profiles. More importantly, this approach is quantitative, i.e. a score is assigned to each co-expression link between two genes in a network, thus allowing us to prioritize candidate meiotic genes and interactions for targeted experiments. Additionally, our study offers a better understanding of meiotic differences between sex and species, suggesting that meiosis is more conserved by sex than by species. Although sexual dimorphism in meiosis is well known, including the differences in timing, outcome, and checkpoint control (Morelli & Cohen 2005, Bowles & Koopman 2007, Kimble & Page 2007, Hunt & Hassold 2008), our genome-wide unbiased approach provides direct evidence on the number of conserved meiotic genes to support sexual dimorphism. Further, our approach allows direct comparison between cross-sex conservation and cross-species conservation. The difference between M_f-H_f and M_m-M_f in identifying known meiotic genes is relatively small, which we believe is partly due to the small number of known meiotic genes that are conserved between human and mouse (~80 genes, indicated as the maximal x-axis value in Fig. 2). However, our conclusion that meiosis is more conserved by sex than by species still holds even when we created precision-coverage curves by sampling every

100 gene pairs (Supplementary Figure 1), by sampling every 200 genes from ranked pairs (Supplementary Figure 2), or by an alternative method of using the minimum to define conserved networks (Supplementary Figure 3). These new approaches indicate that the meiosis conservation by sex is indeed a robust phenomenon.

The known meiotic genes identified from our co-expression networks encode for synaptonemal complex components, meiosis-specific cohesins, recombination proteins, and piRNA complex components. We focused on investigating candidate genes that display direct links with known meiotic genes. Some candidates are supported by published experiments, in which expression was detected in the testis and the ovary. However, other candidates are human disease genes, including tumor suppressors, with no previous associations with meiosis. Interestingly, we know that several genes initially linked to cancer, such as *Mlh1* and *Pms2*, were later identified as meiotic genes because the knockout mice exhibited severe meiotic defects and infertility (Baker *et al.* 1995, 1996). The dual roles of a gene can be explained by shared DNA repair mechanisms. Tumor formation in somatic cells may result from failed DNA damage repair and meiotic recombination in germ cells evolved from somatic DNA repair mechanisms. Therefore, some genes may perform related but not necessarily identical functions in somatic and germ cells; mutations in these genes may cause both tumors and meiotic defects. The candidate meiotic genes identified from our study represent valuable targets for further functional characterization.

We focused on microarray data of testis and ovary tissues from human and mouse. Although it is possible to obtain purified germ cell populations in the male using physical separation methods, it is extremely difficult to isolate germ cells in the female due to the size of the fetal ovary and the source of human tissues. Consequently, microarray data on purified meiotic populations are only available in the male (Shima *et al.* 2004, Chalmel *et al.* 2007), which makes cross-sex inference impossible. One study used isolated male germ cells to characterize conserved expression in human and rodents (Chalmel *et al.* 2007). A clustering method identified 217 potential meiotic genes showing similar expression in male mouse and rat. The precision of identifying known mouse meiotic genes from the 217 genes is 3.2%, comparable to the performance of top 200 gene pairs in our three networks. The advantage of our approach is the capability of prioritizing candidate genes based on the quantitative co-expression links with known meiotic genes. Our study focused on identifying conserved meiotic genes. The limitation of the approach is the possibility of missing sex-specific and species-specific meiotic genes. However, the knowledge of mammalian meiosis is extremely limited, and functional characterization of meiotic genes using animal models is

expensive and time-consuming. Therefore, the current priority is to improve the prediction accuracy on candidate meiotic genes. Our approach fits well with this priority.

In summary, our study identified conserved meiotic programs and predicted novel meiotic genes. This information will enable us to better understand abnormal meiotic processes. The diversity and amount of genomic data will continue to grow, and our approach provides a flexible means by which future data can be integrated to generate new knowledge on mammalian meiosis.

Materials and Methods

Genes conserved between mouse and human

Orthologous clusters of human and mouse genes were downloaded from the database InParanoid (Ostlund *et al.* 2010). InParanoid provides a robust method for detecting orthologous relationships between two species because multiple alignments and phylogenetic trees – error-prone steps in classical ortholog detection – are bypassed. Ortholog clusters in InParanoid are seeded with orthologs based on reciprocal best match between two species, after which inparalogs are added. Only seed orthologs, the most confident genes conserved between mouse and human, were kept for our analysis. We termed these conserved genes MHs. A total of 16 376 MHs were identified, covering ~77% of human genes and 71% of mouse genes. Most MHs contain a single gene from each species, and each gene was assigned to only one MH (Table 1).

Gene co-expression in meiotic prophase

To investigate gene co-expression during mammalian meiotic prophase, we selected three time series microarray studies on mouse postnatal testis (GSE12769), mouse embryonic ovary (GSE6916), and human fetal ovary (GSE15431; Shima *et al.* 2004, Small *et al.* 2005, Houmard *et al.* 2009). Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA, USA) was used for both male and female mouse studies; human Genome U133A Plus 2.0 Array (Affymetrix) was used for the female human study. Time series microarray data are currently unavailable for the human male due to the difficulties of retrieving juvenile testis samples. Only time points within meiotic prophase were included in our analysis, which are 6, 8, 10, and 14 days *post partum* for the male mouse; 11.5, 12.5, 14.5, and 16.5 days *post coitum* for the female mouse; and 9.1, 9.6, 11, 12, 12.9, 13.6, 13.9, 14.4, 16.1, 16.4, 16.9, 17.1, and 18.1 weeks of gestation for the female human. Microarray data were normalized as described in the original studies. Gene signal intensities were obtained by averaging signals of corresponding probes. The maximum signal intensity of each gene was identified across prophase in each microarray study. The top 90% of highly expressed genes based on the maximum signal intensity were kept for co-expression analysis. This criterion prevents the introduction of very lowly expressed genes into the gene co-expression network, as their signals are often dominated by noise.

We calculated the Pearson correlation coefficient r_{xy} to assess co-expression of each MH gene pair across prophase in each microarray study separately:

$$r_{xy} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{(n-1)S_x S_y},$$

where x and y are expression data vector of length n for two genes, $\bar{x}$ and $\bar{y}$ are means, and S_x and S_y are s.d. If an MH contains more than one gene in a species, Pearson correlations for MH gene pairs were computed by averaging multiple gene pairs' Pearson correlations. The correlation coefficients r_{xy} were Fisher's z' -transformed, $z' = 0.5 \times (\ln(1 + r_{xy}) - \ln(1 - r_{xy}))$, to ensure normal distribution of correlation coefficients in each microarray study. The Fisher's z' were further converted into standard z -scores, $z = (z' - \bar{z}')/s_{z'}$, where $\bar{z}'$ is the mean and $s_{z'}$ is the s.d. over all MH pairs for each microarray study. This step ensures comparable standard normal distribution across species and sex.

Conserved co-expression networks of mammalian meiotic prophase

To identify conserved co-expression gene pairs, we averaged standard z -scores across species and sex to obtain the average z -score for each MH gene pair. Obtaining average z -scores across species and sex demands a large computer memory because ~100 000 000 pairs need to be considered from each microarray study. We designed an efficient 'divide and conquer' algorithm to accomplish this calculation on a standard desktop computer. A divide and conquer algorithm works by breaking down a problem into a number of sub-problems until they become simple enough to be solved directly. The solutions to the sub-problems are then combined to give a solution to the original problem. Using the algorithm we designed, the calculation of the two-species networks (M_m-M_f and M_f-H_f) was completed in 1 h, while the calculation of the three-species network ($M_m-M_f-H_f$) was completed in 3 h (iMac with 4 GB memory and 3.06 GHz Intel Core 2 Duo processor). The pseudo-code for the algorithm is provided below.

Input:

- List 1: all possible MH gene pairs (16 376 MH genes $\times$ 16 375 MH genes/2)
- List 2: MH gene pairs with male mouse z -scores
- List 3: MH gene pairs with female mouse z -scores
- List 4: MH gene pairs with female human z -scores.

Divide List 1 into sub-lists so that the computer memory can store one sub-list

For each sub-list

- For each MH gene pair in the sub-list
 - Obtain standard z -scores of the pair from List 2 to 4
 - Obtain the average z -score of the pair
 - Delete the pair from List 2 to 4

Output:

- A list of MH gene pairs with average z -scores.

Randomization of co-expression networks

An observed network was obtained by sorting MH gene pairs in descending order of average z-score. Randomizing the order of MH pairs generated a randomized network. This process was repeated 100 times to produce 100 randomized networks. Results from the average over 100 randomizations were described.

GO term enrichment

We used significantly enriched GO terms to describe common functions of a gene set. To determine whether a GO term is enriched in a gene set, we calculated the *P* value from a hypergeometric distribution:

$$P(X \geq k) = \sum_{x=k}^{\min(m,n)} \frac{\binom{m}{x} \binom{N-m}{n-x}}{\binom{N}{n}}.$$

In this equation, *N* is the total number of genes in a co-expression network, *m* is the number of genes in the network annotated by the GO term, *n* is the number of genes in the set (for example, genes in top 200 MH gene pairs), and *k* is the number of genes in the set and annotated by the GO term.

Semantic similarity

The calculation of semantic similarity for gene pairs was described in detail by Lord *et al.* (2003). Briefly, each GO term in the GO hierarchy is assigned a probability, corresponding to the chance of a concept occurring on this term or its children. Thus, the probability increases as we move to the top of the GO hierarchy, where the probability is equal to 1. A pair of genes may be co-annotated by more than one GO term. To calculate the semantic similarity for a pair of genes, the shared GO term with the minimal probability will be identified. The similarity score is defined as the negative log₁₀ of the minimal probability. Because each gene pair is associated with a similarity score, an averaged semantic similarity can be calculated for a group of gene pairs to quantify their functional association.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/REP-11-0260>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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