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Genetic modification of iron metabolism in mice affects the gut microbiota

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Abstract The composition of the gut microbiota is affected by environmental factors as well as host genetics. Iron is one of the important elements essential for bacterial growth, thus we hypothesized that changes in host iron homeostasis, may affect the luminal iron content of the gut and thereby the composition of intestinal bacteria. The iron regulatory protein 2 (Irp2) and one of the genes mutated in hereditary hemochromatosis Hfe , are both proteins involved in the regulation of systemic iron homeostasis. To test our hypothesis, fecal metal content and a selected spectrum of the fecal microbiota were analyzed from *Hfe*−/−, *Irp2*−/− and their wild type control mice. Elevated levels of iron as well as other minerals in feces of *Irp2*−/− mice compared to wild type and *Hfe*−/− mice were observed. Interestingly significant variation in the general fecal-bacterial population-patterns was observed between *Irp2*−/− and *Hfe*−/− mice. Furthermore the relative abundance of five species, mainly lactic acid bacteria, was significantly different among the mouse lines. *Lactobacillus (L.) murinus* and *L. intestinalis* were highly abundant in *Irp2*−/− mice, *Enterococcus faecium* species cluster and a species most similar to Olsenella were highly abundant in *Hfe*-/- mice and *L. johnsonii* was highly abundant in the wild type mice. These results suggest that deletion of iron metabolism genes in the mouse host affects the composition of its intestinal bacteria. Further studying the relationship between gut microbiota and genetic mutations affecting systemic iron metabolism in human should lead to clinical implications.

Keywords Iron · Gut microbiota · Mineral absorption · Hemochromatosis · Iron regulatory protein 2 (IRP2)

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Introduction

Iron is an essential nutrient for nearly all organisms that is prone to generate reactive oxygen species in its ferrous Fe^{2+} form and has an extremely low solubility at physiologic pH in its ferric Fe^{3+} form (Andrews et al. 2003). These characteristics pose a challenge to biological systems, which protect themselves from the reactive iron by tightly regulating acquisition and by binding iron to proteins for safe transport and storage. On the other hand, because of the low solubility of environmental oxidized iron, organisms had to develop strategies to efficiently acquire and compete on the available sources. In many ecosystems iron is the growth-limiting nutrient (Coale et al. 2004; Behrenfeld et al. 2006). Therefore, the efficiency of

bacterial species to acquire iron is playing a central role in the abundance of the specific species and the equilibrium that is formed within a population.

In the mammalian gastrointestinal tract only 5–20 % of ingested iron is absorbed, depending on the iron status of the organism and residual iron reaches the intestinal microbiota. Bacteria have developed various well-regulated strategies to acquire iron. A common system for iron acquisition is through secreted high affinity iron binding molecules named siderophores. Bacteria bind their own or their neighbor's siderophores which transport iron across bacterial cell walls and membranes (Andrews et al. 2003; Braun and Hantke 2011). In a mixed bacterial population, such as the gut microbiota, the availability of iron directly affects the composition of the population (Dostal et al. 2012), and the metabolic state of the bacterium. In addition iron level is directly connected to virulence of many pathogens (Kortman et al. 2012; Wyckoff et al. 2007; Klein and Lewinson 2011; Beasley et al. 2011).

The gut microbiota, consists of approximately 1,000 bacterial species (Qin et al. 2010), plays an important role in normal gut functioning and is important for maintaining the host-organism in good health (Mai and Draganov 2009; Ashida et al. 2011). Recent studies have shown a tight connection between the composition of the gut microbiota and different health disorders, such as inflammatory bowel diseases, atopic diseases, obesity, metabolic syndrome, intestinal cancers and diabetes (Barbas et al. 2009; Garrett et al. 2010; Lionetti et al. 2010; Qin et al. 2010; Turnbaugh et al. 2010; Vijay-Kumar et al. 2010; Wen et al. 2008). The gut microbiota composition was recently found to be host-specific (Costello et al. 2009; Dethlefsen et al. 2007; Eckburg et al. 2005) and is a result of variations in host genetics (Benson et al. 2010; Buhnik-Rosenblau et al. 2011; Khachatryan et al. 2008; Spor et al. 2011; Zoetendal et al. 2001a; Zoetendal et al. 2001b) and environmental factors, such as dietary components including metal ions (Blaut 2002; Benoni et al. 1993; Dostal et al. 2012; Spor et al. 2011; Mshvildadze et al. 2008; Turnbaugh et al. 2009). Changes of the metabolic state of epithelial cells affect the intestinal homeostasis (Kaser et al. 2011) that in turn may lead to a host specific equilibrium of the bacterial population.

Mutations in the *Hfe* gene can lead to hereditary hemochromatosis, a common genetic disorder of iron overload (Gan et al. 2011; Babitt and Lin 2011). Mutations in *Hfe* affect the iron regulatory hormone hepcidin and its downstream target, the iron exporter ferroportin (Camaschella and Poggiali 2011; Nemeth et al. 2004). Ferroportin may be a general transporter for transition metals (Brissot et al. 2010) and therefore we hypothesized that deletion of *Hfe* in a murine model may affect intestinal epithelial and/or fecal metal content. In addition mice with a targeted deletion of the iron regulatory protein 2 (Irp2) accumulated iron in their intestinal epithelial cells. Levels of both, the apical divalent metal transporter 1 (Dmt1) and the basolateral ferroportin are affected in these cells by the *Irp2* deletion (LaVaute et al. 2001), suggesting that also in these mice luminal and epithelial intestinal metal homeostasis is altered.

Base on this data we hypothesized that genetic modifications of iron metabolism that affect epithelial metal transport may influence the composition of residue intestinal content and/or the metabolic state of the epithelial cell, which may in turn affect intestinal microbiota. Therefore we compared the content of fecal metal- and non-metal-minerals of wild type (WT), *Hfe*−/− and *Irp2*−/−mice and found that many minerals were elevated in the feces of *Irp2*−/− mice. Comparing the profiles of culturable bacterial subpopulations revealed significantly different composition of the *Irp2*−/− gut bacterial population, compared to the *Hfe*−/− mice. Further, we identified several species that their abundance was specifically affected by the genetic modulations of iron homeostasis.

Materials and methods

Mouse lines

All mice were of a C57Bl/6J background. Breedings were approved by the Technion Animal Ethics Committee, Haifa, Israel. *Irp2*−/− mice were a generous gift of Tracey Rouault (Molecular Medicine Program, National Institute of Child Health and Human Development, NIH, Bethesda, MD USA). *Hfe*−/− mice were a generous gift of Joanne Levy (deceased) and Nancy Andrews (Pediatrics and Pharmacology and Cancer Biology, Duke University, Durham, North Carolina, USA). Mice were housed under specific-pathogen-free conditions in individually ventilated

cages. Autoclaved food (Teklad Certified Global 18 % Protein Rodent Diet, Harlan Laboratories Ltd.) and sterilized deionized water were provided ad libitum. Cages were held in a room with 12/12 h light/dark cycle, 24 ± 1 °C, 60–70 % humidity and centrally controlled ventilation with HEPA filters (75 air change/h). Feces samples were collected from female mice aged 3–4 months. Feces were collected from each individual mouse separately. For the tRFLP analysis, feces of similar size were collected from three individuals of the same mouse line and were pooled. The sampling was performed in two biological replications, in which each mouse group was sampled twice and the bacterial suspension from each pooled feces sample was cultivated and grown in duplicates, eight technical replications in all.

Fecal metal quantification by inductively coupled plasma mass spectrometry (ICP-MS)

Fecal samples were analytically weighed and each two samples were digested with 200 µl of 70 % nitric acid in 60 °C for 30 min. 200 µl of 30 % H_2O_2 was added to each digested sample, and tubes were re-heated to 60 °C for another 30 min. Samples were diluted to 1 % nitric acid with ddH_2O, filtered and analyzed in an ICP Spectrometer, iCAP 6000 Series (Thermo Scientific). Data was analyzed using ANOVA followed by means separation with the Student's *t* test using the statistical software JMP 8 (2008 version, SAS Institute Inc., Ca).

Isolation of bacterial DNA from feces

Feces samples were suspended in 0.1 M sodium phosphate buffer pH 7 to a final concentration of 10 % (w/v) by vigorous vortexing, followed by centrifugation at 1,500×*g* at 4 °C for 5 min. The supernatant containing the bacterial suspension was transferred to a clean tube, and 100 µl of bacterial suspension was spread on Difco m-*Enterococcus* agar plates (BD, Sparks, MD, USA) and Difco Brain Heart Infusion (BHI) agar plates (BD, Sparks, MD, USA) in four dilutions (non-diluted sample, 1:10, 1:100 and 1:1,000); these were grown under anaerobic conditions at 37 °C for 48 h. Crude DNA extraction procedure was performed as follows: cells from a loopfull of non-diluted fecal-bacterial populations were suspended in 70 % ethanol (1 ml) by vigorous vortexing, 33 µl of sodium acetate (3 M, pH 5.2) was added and the samples were incubated at −80 °C for 20 min followed by centrifugation at 12,000×*g* for 15 min. The supernatant was removed and the pellet was dissolved in 30 µl 0.1× Tris EDTA (TE). The crude DNA was diluted tenfold in ddH_2O and stored at −20 °C. Also single colonies were used directly for PCR in order to identify of the bacterial species by specific tRFLP and 16S rRNA gene sequencing (see below).

tRFLP of fecal-bacterial populations

16S rDNA of fecal-bacterial populations was amplified using 27F-FAM and 1492R primers (Sakamoto et al. 2003), at an annealing temperature of 60 °C. The PCR products were purified by ethanol precipitation and dissolved in ddH_2O. The purified PCR product (1,000 ng) was digested with 20 U of Msp1 restriction enzyme (New England Biolabs, Ipswich, MA, USA) in a total volume of 20 µl for 2.25 h at 37 °C followed by 20 min at 65 °C. The digested DNA (50 ng) was loaded into a 3,130 genetic analyzer together with 9 µl of formamide and 0.5 µl of GENESCAN 1200 LIZ size standard (lot 0709012; Applied Biosystems, Foster City, San Mateo, CA, USA) for size determination. The results were analyzed with GeneMapper 4.0 software (Applied Biosystems).

The bacterial species corresponding to each of the main tRFLP peaks were identified by size analysis of the 16S terminal restriction DNA fragments (tRFs) of representative colonies and followed by 16S rDNA sequencing and by in silico t-RFLP analysis (http://insilico.ehu.es/T-RFLP/)(Bikandi et al. 2004).

PCR for tRFLP and 16S rRNA gene sequencing

Each PCR mixture contained 0.2 mM deoxynucleoside triphosphates, 0.4 µM forward and reverse primers, 0.02 U of *Taq* polymerase (SuperNova, JMR Holding, Kent, England) per µl, 1× reaction buffer (containing 1.5 mM $MgCl_2$) and 10 µl of ten-fold diluted crude DNA or, alternatively, spiked cells from a colony (see above). The reactions were carried out in a Veriti® 96-Well Thermal Cycler (Applied Biosystems) as follows: 95 °C for 3 min; 30 cycles of 30 s at 95 °C, 30 s at the annealing temperature, and 90 s at 72 °C; 10 min at 72 °C; cooling to 12 °C. PCR amplification products were verified by gel

(1.2 % Agar) electrophoresis and visualized by UV fluorescence.

DNA sequencing

PCR amplification products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Purified DNA (20–50 ng) was sequenced on both strands using a BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems) and loaded into the ABI 3130 genetic analyzer. Results were analyzed with SeqScape 2.5 software (Applied Biosystems) and DNA sequencing analysis 5.2 software (Applied Biosystems).

Data (tRFLP) and statistical analyses

Relative abundance was calculated for each tRFLP peak by dividing the peak area with the total area of all peaks of the analyzed sample. A combined analysis was used to evaluate the similarity of the microbial sub-populations between the mouse lines. This was performed by principal component analysis (PCA) of the combined tRFLP relative abundance data obtained from the two growth media using the software "PAST" (Hammer et al. 2001).

The relative abundance of each main tRFLP peak was individually compared between the mouse lines by performing ANOVA followed by means separation with the Tukey's honestly significant difference (HSD) test using the statistical software JMP 8 (2008 version, SAS Institute Inc., Ca). The obtained 16S-rDNA sequences were compared to all available sequences using the NCBI BLAST algorithm for species identification.

Results

Elemental analysis of fecal content of *Irp2*−/−, *Hfe*−/− and WT C57BL/6J mice

We hypothesized that the feces-composition of *Irp2*−/− and *Hfe*−/− mice may differ from WT mice due to the effect of the deletion of *Irp2* or *Hfe* genes on iron transporters, and the iron retention in intestinal epithelial cells of *Irp2*−/−mice. To test this fecal metal and non-metal elements were analyzed by ICP-MS. Most tested minerals that were above detection limit were significantly elevated in the feces of *Irp2*−/− mice compared to both WT and *Hfe*−/− mice, which were similar (Fig. 1a, b). In particular, iron showed the most significant elevation ($p = 0.0017$) in feces of the *Irp2*−/− mice. Ni, Pb, Si, Ag, Cd, Co, Mo, Ba and Cr were below the detection limit of the instrument.

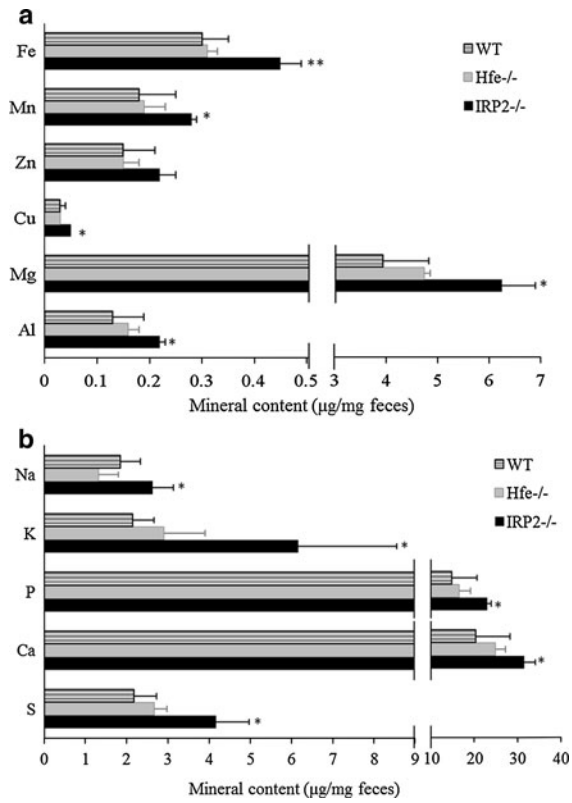

Fig. 1 Mineral concentration in mouse-feces. Feces from *Irp2*−/− ($n = 4$), *Hfe*−/− ($n = 3$), and WT ($n = 3$) mice were analyzed by ICP-MS. Metal concentration (**a**) and concentration of other minerals (**b**) are shown. Each bar represents the mean ± SD. Statistical significance was analyzed by ANOVA followed by means separation with the Student's *t* test * $p < 0.05$, ** $p < 0.005$

Fecal sub-population profiles of *Irp2*−/− *Hfe*−/− and WT C57BL/6J mice

Fecal-bacterial sub-populations of the various mouse lines were analyzed by growing the total microbiota on two media, BHI and m-*Enterococcus*. BHI is suitable for cultivation of a wide variety of microorganisms, whereas m-*Enterococcus* agar is highly selective for

some bacterial species, mainly *Enterococci*, which is one of the main genera belonging to the lactic acid bacteria (LAB).

tRFLP analysis of the bacterial sub-population selected on each growth medium revealed reproducible patterns within each of the *Irp2*−/−, *Hfe*−/− and WT C57BL/6J mouse lines (Fig. 2a, b). To assess variations of the fecal-bacterial populations among the three mouse lines, principal component analysis (PCA) was performed based on the tRFLP results obtained from the two growth media together (Fig. 3). The analysis revealed significantly different ($p < 0.05$) fecal-bacterial populations between *Irp2*−/− and *Hfe*−/− mice, in contrast to statistically non-significant differences between the bacterial population of the WT compared to each of the knock out lines. Similar results were obtained by PCA of the tRFLP data of each of the growth media separately (data not shown).

Individual bacterial species in the feces of *Irp2*−/−, *Hfe*−/− and WT C57BL/6J mice

Following characterization of the selected microbiota at the population level, we focused on individual bacterial species that were the main compositors of each of the bacterial sub-populations. The bacterial species corresponding to each of the main tRFLP

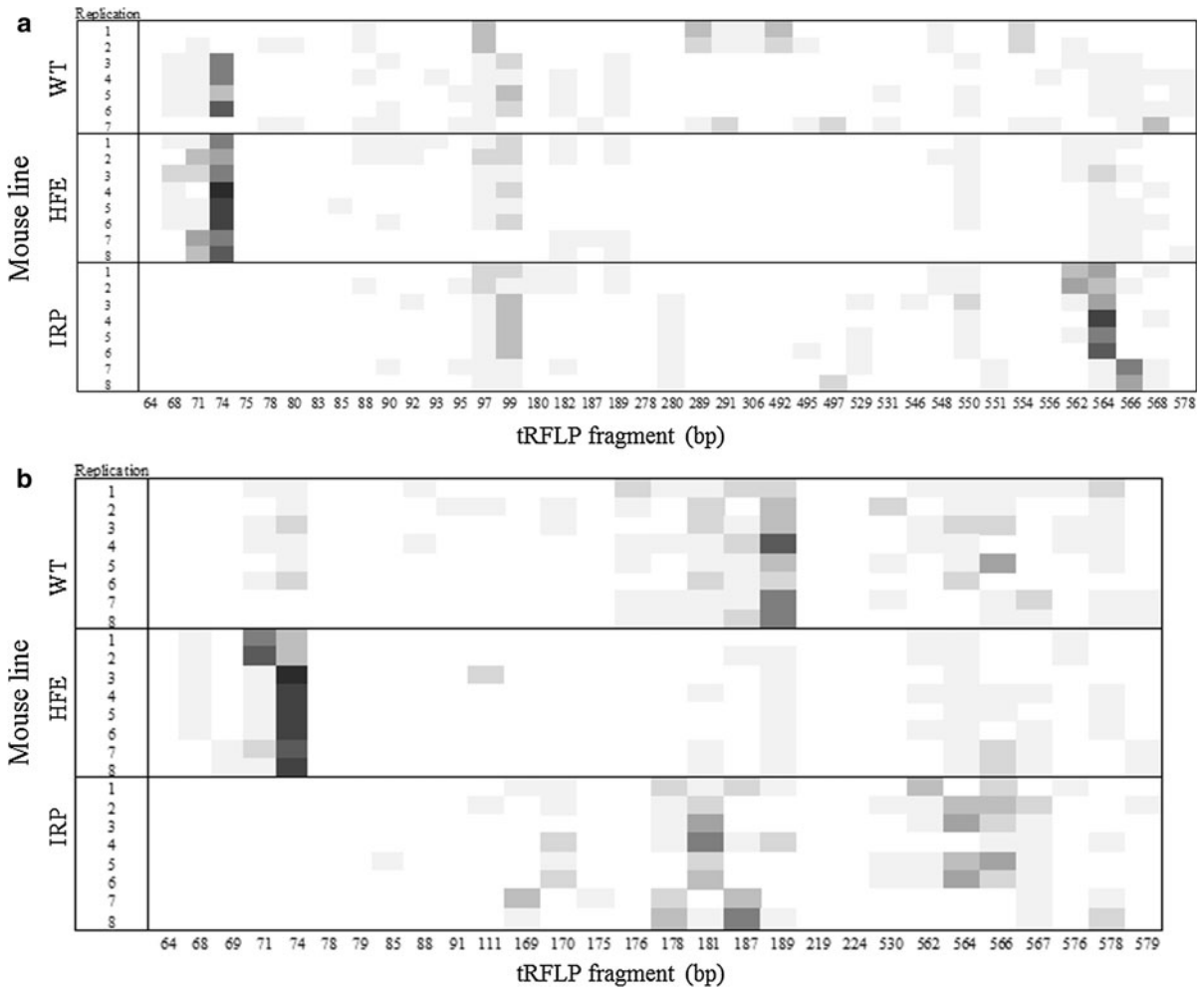

Fig. 2 Relative abundances of tRFLP fragments representing the gut bacterial populations of WT, *Hfe*−/− and *Irp2*−/− mice. Fecal bacterial populations were selected on: **a** brain heart infusion (BHI) agar **b** m-*Enterococcus* agar. Sizes of tRFLP fragments are given in base-pairs (bp). *Shading* represents relative abundance, divided into eight levels, with *darker shading* indicating higher abundance

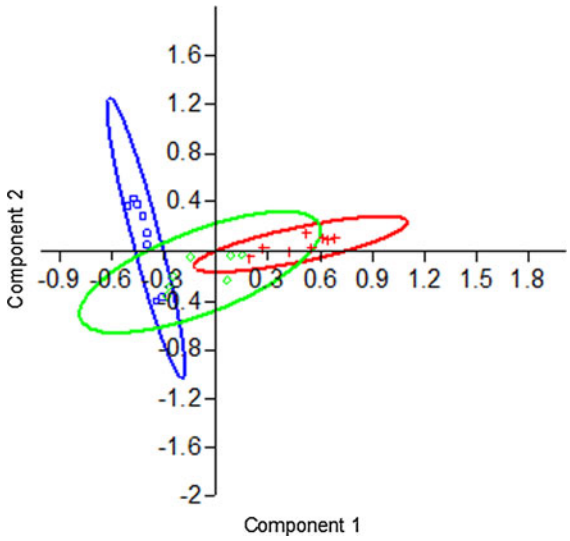

Fig. 3 Principal component analysis (PCA) of the fecal bacterial population profiles grown on BHI- or m-*Enterococcus* agar plates. *Green circles*, *red crosses* and *blue squares* represent the fecal bacterial population profiles originated from WT, *Hfe*−/− and *Irp2*−/− mice respectively. The components PC1 and PC2 explain 62 % of the variance. 95 % ellipses are indicated

peaks were identified by analyzing the 16S terminal restriction DNA fragments (tRFs) of representative colonies followed by 16S rDNA sequencing.

In the BHI growth medium, the main 74 bp peak was accompanied with peaks of 68 and 71 bp (Fig. 2a). These peaks all correlated with an unidentified bacterial species with highest similarity to Olsenella sp. The 564 and 566 bp peaks were both identified as *L. murinus* (Fig. 2a). In the m-*Enterococcus* medium, the 74 bp tRFLP-peak was found to represent species belonging to the *Enterococcus faecium* cluster, the 181 bp peak—*L. intestinalis*, the 189 bp peak—*L. johnsonii* and the 564 and 566 bp peaks both represented *Enterococcus faecalis* (Fig. 2b).

In five out of these six bacterial species, significantly different abundances were detected among the mouse lines as determined by ANOVA. *Irp2*−/− mice had higher levels of *L. intestinalis* compared to *Hfe*−/− mice and higher levels of *L. murinus* compared to both *Hfe*−/− and WT mice; *Hfe*−/− mice had higher levels of the *E. faecium* species cluster and of an unidentified species with highest similarity to *Olsenella* sp. compared to both WT and *Irp2*−/− mice; and the WT mice presented higher levels of *L. johnsonii* compared to the two mutant mouse lines (Fig. 4).

Taken together, the deletions of the *Irp2* and *Hfe* genes, which affect the iron homeostasis of the host, influenced the composition of its fecal microbiota. Deletion of the *Irp2* gene also caused a significant elevation of the fecal mineral content.

Discussion

The gut microbiota is composed of thousands of bacterial species, affected by many environmental and genetic factors (Jakobsson et al. 2010; Ley et al. 2008; Mshvildadze et al. 2008; Turnbaugh et al. 2009; Benson et al. 2010; Esworthy et al. 2010; Spor et al. 2011). Iron is one of the important factors essential for bacterial growth, and a target for commensal and virulent bacteria as well as the host that compete on the same mineral.

It was previously shown that dietary induced alteration in luminal iron significantly changed the gut microbiota in rats (Benoni et al. 1993; Dostal et al. 2012). Here we show that the intestinal microbiota of mice is affected by genetic deletion of the *Hfe* and *Irp2* genes, which both affect the systemic and intestinal epithelial iron homeostasis and have roles in the regulation of systemic and cellular iron metabolism (Babitt and Lin 2011; Rouault 2006).

Fecal bacteria reflect a combination of shed mucosal bacteria and a separate non-adherent luminal population (Eckburg et al. 2005) and were assigned to represent the host specific (Eckburg et al. 2005; Benson et al. 2010) and stable (Zoetendal et al. 1998) gut microbiota. Therefore we chose to analyze the gut microbiota in our murine models by fecal sampling and to focus on two sub-populations of the fecal microbiota, selectively grown on different media. BHI agar enables the growth of a wide bacterial spectrum, while m-*Enterococcus* agar enables the growth of a limited number of bacterial species belonging to the LAB, a bacterial group, which contains many probiotic strains. The bacterial population selected on the m-*Enterococcus* agar was previously found to differ among genetic mouse lines (Buhnik-Rosenblau et al. 2011).

Three mouse lines were tested, WT-C57BL/6J and mice with targeted deletions of the *Irp2* and *Hfe* genes both on the C57BL/6J background. Being genetically uniform, each mouse line presented a highly reproducible fecal-bacterial profile, as analyzed by tRFLP (Fig. 2a, b). Such reproducibility of the fecal-bacterial

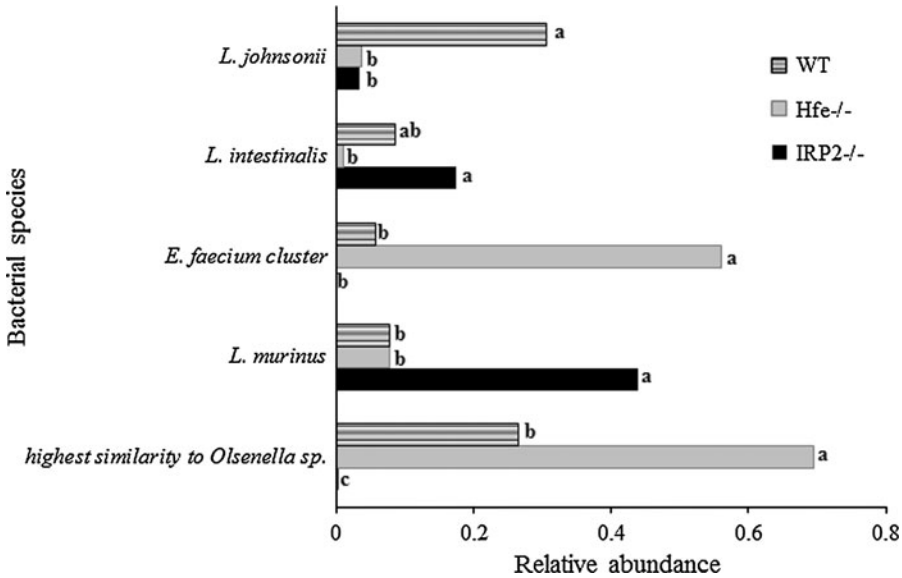

Fig. 4 Levels of *Lactobacillus (L.) murinus*, *L. intestinalis*, *L. johnsonii*, *E. faecium* species cluster and an unidentified species most similar to *Olsenella* sp. in fecal samples of C57BL/6 WT, *Hfe*−/− and *Irp2*−/− mice. Levels are expressed as the relative abundances of the corresponding tRFLP peak from the total bacterial sub-population, and are given as average values of eight replicates. Columns headed by different letters are significantly different within each bacterial species at $\alpha = 0.05$ by ANOVA followed by Tukey HSD test

populations within the same genotype kept under the same conditions was similarly demonstrated in previous studies (Buhnik-Rosenblau et al. 2011; Zoetendal et al. 1998). In contrast to the high conservation within lines, wide differences were demonstrated in the bacterial profiles between the two mutant lines, *Irp2*−/− and *Hfe*−/−, which presented significantly distinct bacterial populations according to principal component analysis (PCA) results (Fig. 3). This finding may indicate that the deletions of each of those genes have created new intestinal microenvironments to which the bacterial populations of the WT mice adapted. However, the bacterial profile of the WT mice overlapped with those of each of the mutant lines in the PCA, emphasizing the partial similarity between the fecal-bacterial population of the WT line and each of the mutant lines. These findings support a notion that a higher degree of genetic variation (when comparing the *Irp2*−/− mice to *Hfe*−/− mice) is reflected in a larger variation of bacterial populations.

The apical metal transporter Dmt1 and the basolateral exporter ferroportin are two important transporters involved in duodenal iron uptake. Their levels are reported to be elevated in mice with a deletion of *Usf2*, that are similar to the *Hfe*−/− mice in respect to misregulated and low hepcidin levels (Viatte et al. 2005). In mice with targeted deletions of *Hfe* the background-strain affects Dmt1 and ferroportin levels. Thus not all backgrounds have elevated expression of these transporters (Herrmann et al. 2004), which explains why the reports on their levels are conflicting (Griffiths et al. 2001; Dupic et al. 2002). In a previous study it was shown that in *Irp2*−/− mice, Dmt1 and ferroportin both are elevated (LaVaute et al. 2001) which could lead to increased mineral absorption and decreased fecal mineral content. Surprisingly here we found that many minerals in the feces of *Irp*2−/− mice were elevated and fecal mineral content in *Hfe*−/− mice was not changed. In humans only about 10 % of dietary iron is absorbed, leaving 90 % to fecal excretion. In mice absorption may be slightly higher (Drake et al. 2007) still leaving 80 % of iron in the feces. Therefore even a 50–100 % increase in iron absorption, as might occur in *Hfe*–/– mice, will lead to small changes in the fecal preparations that may be hard to detect but can influence the microbiota in those mice. In contrast the effect of the deletion of *Irp*2 seems to be vigorous enough to be detected in fecal preparations.

While both, deletions of *Irp*2 and *Hfe* may affect Dmt1 and Fpn levels, only *Irp*2−/− mice accumulate ferritin in the duodenal epithelial cells. This may lead to a functional iron deficiency and metabolic malfunction in those cells. The increased fecal mineral content of *Irp*2−/− mice may therefore be due to a general decrease in the epithelial cells ability to absorb, a defect that may dominate the effect of the increased specific transporters. In addition, sloughing of epithelial cells that contain ferritin-iron to the lumen of the intestine may further elevate fecal-iron

concentration in these mice. However, the fact that deletion of the *Hfe* gene affected the bacterial population without changing the fecal mineral content further emphasizes that other factors, such as mineral homeostasis of the epithelial cells can affect the composition of the microbiota. These effects can be on certain subpopulations of bacteria that are in close interaction with the epithelial cells and their mucous coat, and changes in these populations will indirectly affect the equilibrium of the microbiota as a whole.

Following the assessment of the selected microbiota at the population level (Fig. 2), we concentrated on the main bacterial compositors of the two selected subpopulations. Out of the six bacterial species identified, five presented significantly different levels among the mouse lines (Fig. 4), while one species, *E. faecalis*, was present at similar relative abundances. This species was also equally found in the gut microbiota of C57BL/6J and BALB/C mice and may therefore represent a stable species as the other LAB species differed among the two host lines (Buhnik-Rosenblau et al. 2011). The levels of four LAB species, *L. murinus*, *L. intestinalis*, *L. johnsonii*, and of the *E. faecium* species cluster, varied among the lines. The effect of mutations in iron metabolism genes on these species indicates a direct or indirect influence of these genes on the ability of potentially probiotic bacteria to persist in the gut of the host.

Probiotic bacteria confer a health benefit on the host, when administrated in adequate amounts (Pineiro and Stanton 2007). Much interest has developed in recent years in strategies to tilt the gut microbial equilibrium to the benefit of the host. Many probiotic LAB strains have been beneficial in the treatment of a wide range of diseases (Haller et al. 2010; Holubar et al. 2010; Kalliomaki et al. 2010; Lionetti et al. 2010; Wolvers et al. 2010). LAB strains are unusual organisms in that they appear not to require any or very little iron (Pandey et al. 1994; Bruyneel et al. 1989; Imbert and Blondeau 1998). It was therefore of concern that iron fortification will let iron dependent and sometimes pathogenic bacteria out-compete the advantageous LAB. Bailey et al. (2011) recently showed that noradrenaline mediated iron supplementation increased the growth rate and health benefits of some LAB strains. This finding is in agreement with our finding, where *Irp2*−/− mice had elevated fecal iron concentrations and the abundance of two LAB species was elevated as well in these mice (Figs. 1, 4). The higher abundance of *L. intestinalis* and *L. murinus* may be a direct effect of the higher iron availability in the intestinal lumen of *Irp2*−/− mice on the bacterial growth or indirectly due to changes in other bacterial species and their interactions with these two LAB species.

Our results are supported by an increasing number of studies, indicating a role for host genetics in determining its gut microbiota. Some of these studies made use of novel DNA-sequencing techniques that provide a wide view of the gut microbiome (Frank and Pace 2008; Qin et al. 2010). However, using cultivation-based methods enabled us to concentrate on a defined bacterial spectrum and to isolate and further characterize selected bacterial species and strains, including potentially probiotic strains. We show that changes in the host genetics are followed by changes in a specific spectrum of the cultivatable gut microbiota. These shifts are both reflected in the genetics of the host-microbe-hologenome and likely affect the biochemical properties of this one functioning unit (Rosenberg and Zilber-Rosenberg 2011).

In conclusion, our findings suggest that genetic modifications of genes affecting iron metabolism have an influence on the composition of murine fecal-bacterial populations including the composition of potentially probiotic bacterial species. Understanding these processes in general can lead to the development of probiotic products tailored to human populations that carry mutations causing modified iron homeostasis such as hereditary hemochromatosis.

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