



Mice studies disentangle the role of estrogen in gallstone formation

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Articles commented:

- de Bari O, Wang HH, Portincasa P, *et al.* The deletion of the estrogen receptor alpha gene reduces susceptibility to estrogen-induced cholesterol cholelithiasis in female mice. *Biochimica et Biophysica Acta* 2015; 1852: 2161-9.
- de Bari O, Wang TY, Liu M, *et al.* Estrogen induces two distinct cholesterol crystallization pathways by activating ERalpha and GPR30 in female mice. *Journal of Lipid Research* 2015; 56: 1691-700.

Comment:

Gallstone disease represents one of the most commonly encountered diseases in gastroenterology.¹ To facilitate the diagnosis and to help to remember the risk factors of gallstones, medical students are taught the mnemonic '5Fs' (or '7Fs') gallstone rule, which, among others, points to female gender and fertility. Indeed, it has been appreciated that female gender and pregnancies increase gallstone risk.^{2,3} This is commonly contributed to the induction of hepatic cholesterol synthesis and biliary cholesterol secretion by estrogens and the relaxation of the gallbladder by progesterone. Gallbladder walls from cholecystectomized patients showed that estrogen receptors are overexpressed in gallbladders from women with gallstones and that gender differences exist in the coexpression of estrogen and progesterone receptors.⁴ Along this line, gallstone disease is also more prevalent in girls receiving oral contraceptives⁵ as well as in postmenopausal women using estrogen therapy.⁶ Experimental studies in gonadectomized inbred mice showed that the prolithogenic action of estrogens is modulated by estrogen receptor α (ER α), whereas the second receptor ER β is not involved in this process.⁷ To date

however, the exact role of female hormones in the pathogenesis of gallstones has not been fully elucidated. In this respect, two latest studies^{8,9} in mouse models provide novel insights in this topic. In the first study, the authors analysed ER $\alpha^{-/-}$ mice that were ovariectomized to rule out differences in endogenous estrogens.⁸ These animals were fed lithogenic diet (containing 15% butter fat, 1% cholesterol and 0.5% cholic acid) and received 17 β -estradiol (E2), a selective agonist of the estrogen receptors (subcutaneously implanted pellets releasing 6 μ g/day), for 56 days.⁸ The phenotypic characterization of ER $\alpha^{-/-}$ mice demonstrated a decreased prevalence of gallstones in comparison to ovariectomized wild-type mice. In line with this observation, the absence of ER α resulted in decreased biliary cholesterol (and phosphatidylcholine) secretion and lower cholesterol saturation index. Of note, hepatic steady-state mRNA levels of HMG-CoA-reductase (*Hmgcr*) as well as the apical cholesterol transporter *Abcg5/g8* were reduced significantly compared to wild-type controls, in which the negative feedback regulation of cholesterol synthesis was impaired. ER $\alpha^{-/-}$ animals were also characterised by improved gallbladder emptying, which was decreased in wild-type controls, pointing to the role of ER α as a mediator of estrogen-induced impairment of gallbladder contractility.

Further insights into the potential mechanisms of gallstone induction through estrogens come from another study,⁹ in which the effects of a 12 day E2 challenge coupled with lithogenic diet were compared in animals with deleted ER α and GPR30. G protein-coupled receptor (GPR) 30 is another high-affinity membrane-bound estrogen receptor.¹⁰ To investigate the potential lithogenic role of GPR30, three groups of mice were analysed: GPR30 $^{-/-}$, ER $\alpha^{-/-}$ and GPR30 $^{-/-}$ /ER $\alpha^{-/-}$ mice. Immunohistochemical analysis demonstrated that ER α was

expressed mainly in gallbladder smooth muscle cells, whereas GPR30 is expressed predominantly in epithelial (and muscle cells) of gallbladder, indicating that different mechanisms contribute to impaired gallbladder function.⁹ E2 with lithogenic diet resulted in cholesterol supersaturated bile both in GPR30^{-/-} and ER α ^{-/-} animals. Moreover, both groups of single knock-out mice showed decreased gallbladder emptying. In contrast, cholesterol crystallization was absent in bile of GPR30^{-/-}/ER α ^{-/-} double-knock-out mice. Of note, gene expression patterns in gallbladders of these mice (including mucin genes and *Abcg5/g8*) were not changed in comparison to wild-type mice receiving no E2 either. Due to differences in biliary lipid composition, distinct cholesterol crystallization pathways were observed in ER α ^{-/-} mice and GPR30^{-/-} mice, with classic monohydrate and anhydrous cholesterol crystals appearing first, respectively. Interestingly, the later pathway predominates also in ABCB4^{-/-} knock-out mice that represent the mouse model of the low phospholipid-associated cholelithiasis syndrome.¹¹

What is the meaning of these findings? First of all, they further underscore the role of female hormones in the development of gallstones and point to GPR30- and ER α -mediated supersaturation of bile as potential mechanism. Of note, however, further studies are required to dissect the signalling pathways that are involved in these mechanisms. Of note, a recent genome-wide association study in a Latin Chilean population comprising 529 cases (489 women) and 566 controls confirmed the *ABCG5/G8* gene (encoding the hepatobiliary cholesterol transporter) as general susceptibility gene but also revealed an additional highly significant signal inside the *GPR30* gene ($P = 5.8 \times 10^{-6}$) in women.¹² This result is in line with previous genome-wide studies in inbred mice that identified the mouse homolog of *GRP30* as credible candidate for the gallstone susceptibility gene *Lith18*.¹³ These findings taken together indicate that the same mechanisms could drive gallstone formation in the mouse models as well as in humans.

Secondly, based on the new findings we may speculate about the possible role of estrogen receptor antagonists as inhibitors of gallstone formation. However, since estrogen receptors appear to stimulate biliary phospholipid secretion⁸ and are involved in numerous processes beyond bile formation (including for example atherosclerosis¹⁴), it remains an open question whether pharmacologic intervention directly affecting their function would be beneficial for patients at high gallstone risk.

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