Steroidogenic Acute Regulatory Protein (*Star*) Biosynthetic Pathway Bioinformatics Project and Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) Ethical Review

Honors Distinction Project

BIOL 460 Senior Thesis

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Introduction

Progestins, androgens, estrogens, and corticoids are the four significant types of steroid hormones (Zubeldia-Brenner et al., 2016). Steroid hormones are synthesized mainly in the mitochondria and the smooth endoplasmic reticulum of the adrenal cortex, the gonads, and the placenta (Holst et al., 2004). They are lipophilic molecules commonly utilized as chemical messengers by organisms in organizational and activational processes (Holst et al., 2004 & Zubeldia-Brenner et al., 2016). Organizational processes focus on the critical actions of steroid hormones during early development (Zubeldia-Brenner et al., 2016). Activational processes focus on the high levels of steroid hormones experienced in puberty, adulthood, and breeding behavior (Zubeldia-Brenner et al., 2016).

Steroid hormones have a significant influence on a wide array of organizational and activational biological processes experienced by organisms throughout their lives (Zubeldia-Brenner et al., 2016). Biological processes influenced by steroid hormones include development, hypothalamic programming, sexual differentiation, reproductive physiology, behavior, osmoregulation, metabolism, regulation of the hypothalamic-pituitary-gonadal axis, and the hypothalamic-pituitary-adrenal axis (Zubeldia-Brenner et al., 2016). The importance of steroid hormones importance on an organism's ability to function correctly is demonstrated by the conditions that can arise due to steroid hormone Insufficiency (Zubeldia-Brenner et al., 2016). Insufficiency in steroid hormones can result in cancer, steroid insensitivity, abnormal fertility, endocrine alterations, and adrenal insufficiency (Genecards CYP11A1, n.d. & Zubeldia-Brenner et al., 2016).

Steroid hormone insufficiency can also be caused by a mutation in the rate-limiting step of the synthesis of steroid hormones (Zubeldia-Brenner et al., 2016). The Steroidogenic Acute Regulatory Protein (STAR) controls the rate-limiting step of steroid hormone synthesis through the mediation of cholesterol transfer from the cytoplasm into the mitochondria (Fig. 1) (OMIM # 201710, n.d. & Zubeldia-Brenner et al., 2016). When the STAR gene is mutated within humans, it is associated with Lipoid Congenital Adrenal Hyperplasia (LCAH) (OMIM # 201710, n.d.). LCAH is the most severe disorder of steroid hormone biosynthesis resulting in a severe deficiency of steroids as well as cellular damaged from an accumulation of cholesterol esters (UniProt ConsortiumEuropean Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics, n.d.). Most individuals impacted by LCAH are female phenotypically. They typically die from severe salt-losing syndrome if not treated within early infancy (UniProt ConsortiumEuropean Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics, n.d.). The goal of this bioinformatic analysis of the STAR biosynthetic pathway and the proteins involved in the rate-limiting step is to provide a deeper understanding of the functioning of that pathway and which proteins are critical to preventing the genetic loss of steroidogenesis resulting in LCAH.

Image of the STAR biosynthetic pathway for general reference:

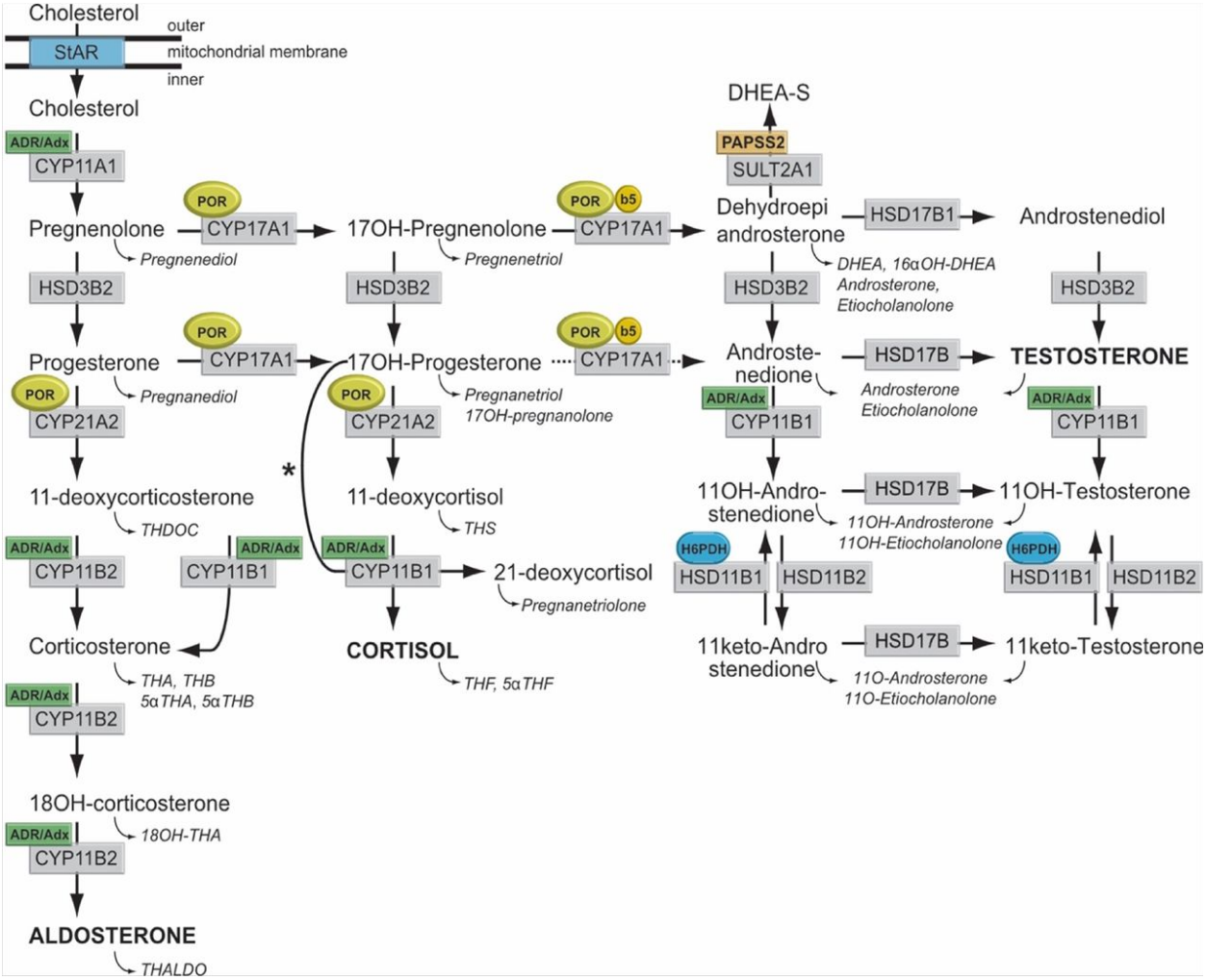

Figure 1. STAR biosynthetic pathway. Taken from Bacila et al., 2019

Star **Biosynthetic Pathway Gene Overview**

CYP11A1

CYP11A1 is the first enzyme within the *STAR* biosynthetic pathway that transforms cholesterol into pregnenolone; it can then be involved in producing aldosterone, cortisol, or testosterone (NCBI CYP11A1 n.d.). This conversion is a rate-limiting step in the *STAR* biosynthetic pathway. CYP11A1 in humans is located on chromosome 15, has a total of ten exons, and encodes for proteins apart of the cytochrome P450 family, specifically the 11 subfamily (NCBI CYP11A1 n.d.). Proteins within the cytochrome P450 family are commonly involved in drug metabolism and the synthesis of cholesterol, steroids, and other lipids in humans (NCBI CYP11A1 n.d.). CYP11A1 is also commonly referred to as CYP11A, CYPXIA1, and P450SCC. Diseases associated with CYP11A1 deficiency in humans are Adrenal Insufficiency, Congenital, With 46, X.Y. Sex Reversal, Partial Or Complete and Inherited Isolated Adrenal Insufficiency (Genecards CYP11A1 n.d. & W.L. et al., n.d.). The general expression of CYP11A1 is observed within the fetal heart, fetal liver, secretory glands, and the reproductive system (Genecards CYP11A1 n.d. & W.L. et al., n.d.).

In zebrafish, *cyp11a1* is located on chromosome 25 (*ZFIN cyp11a1*, n.d.). *Cyp11a1* is involved in C21-steroid hormone biosynthesis, epiboly, and regulation of microtubule polymerization (*ZFIN cyp11a1*, n.d.). *Cyp11a1* is expressed during sexual differentiation in zebrafish embryos, in the adult brain, testicular Leydig cells, in the cytoplasm of oocytes, and the granulosa/theca layer of the ovary (Hsu et al., 2002). *Cyp11a1* is dependent on the nuclear receptor Ff1b with possibly two FF1 response elements bound to it (Quek and Chan, 2009).

Relevant primary literature:

Hsu, H.J., Hsiao, P., Kuo, M.W., and Chung, B.C. (2002) Expression of zebrafish cyp11a1 as a maternal transcript and in yolk syncytial layer. Gene expression patterns : GEP. 2(3-4):219-222.

Quek, S.I., and Chan, W.K. (2009) Transcriptional activation of zebrafish cyp11a1 promoter is dependent on the nuclear receptor Ff1b. Journal of molecular endocrinology. 43(3):121-130.

HSD3B2

HSD3B2 is a gene involved in producing aldosterone, cortisol, and testosterone in the STAR biosynthetic pathway. HSD3B2 encodes for an enzyme involved in the catalyzation of the oxidative conversion of ketosteroids (NCBI HSD3B2 n.d). Deficiency in HSD3B2 in humans is linked to Congenital Adrenal Hyperplasia and 3-Beta-Hydroxysteroid Dehydrogenase Deficiency (Genecards HSD3B2 n.d.). HSD3B2 deficiency has been linked to nonsense and frameshift mutations (Genecards HSD3B2 n.d.). In humans, HSD3B2 has four exons, is located on chromosome 38, and comprises 8,109 base pairs (OMIM * 613890 n.d. & Genecards HSD3B2 n.d.). HSD3B2 is situated in the mitochondria and endoplasmic reticulum (Genecards HSD3B2 n.d.). The protein from HSD3B2 in humans is mainly expressed in the frontal cortex, rectum, adrenal glands, urinary bladder, and reproductive organs (Genecards HSD3B2 n.d.).

In Zebrafish, *hsd3b2* is located on chromosome 20 (*ZFIN hsd3b2*, n.d.). *Hsd3b2* is mainly expressed in the brain, gonads, kidney, and yolk syncytial layer (*ZFIN hsd3b2*, n.d.). There are two forms of *hsd3b* genes within Zebrafish, *hsd3b1*, and *hsd3b2* (Lin et al., 2015). *Hsd3b1* more closely resembles the human HSD3B2 gene (Line et al., 2015). Zebrafish knockouts for *hsd3b1* resemble human HSD3B2 deficiency with interrenal and anterior pituitary expansion as well as increased pigmentation (Lin et al., 2015).

Within Zebrafish, *hsd3b2* is only expressed for the first 30 hours post fertilization (Line et al., 2015).

Relevant primary literature:

Lin, J. C., Hu, S., Ho, P. H., Hsu, H. J., Postlethwait, J. H., & Chung, B. C. (2015). Two Zebrafish hsd3b Genes Are Distinct in Function, Expression, and Evolution. Endocrinology, 156(8), 2854–2862. https://doi.org/10.1210/en.2014-1584

CYP21A2

CYP21A2 is involved in the production of aldosterone and cortisol through the STAR biosynthetic pathway. It is also known as CA21H, CAH1, CPS1, CYP21, CYP21B, and P450c21B (NCBI Cyp21a2 n.d.). The gene coding for CYP21A2 is a member of the cytochrome P450 superfamily (Genecards CYP21A2 n.d.). In humans, It is located on chromosome 6 (NCBI Cyp21a2 n.d.) and has ten exons (OMIM * 613815 n.d.). Deficiency in CYP21A2 in humans is associated with Congenital Adrenal Hyperplasia (Genecards CYP21A2 n.d.).

The protein encoded by the CYP21A2 gene is specifically used to catalyze the 21-hydroxylation of steroids (Genecards CYP21A2 n.d.). Adrenal synthesis of mineralocorticoid and glucocorticoids depend on this catalyzation of 21-hydroxylation from CPY21A2 (Genecards CYP21A2 n.d.). Proteins encoded by CYP21A2 are mainly expressed in the adrenal glands and the cervix in humans (Genecards CYP21A2 n.d.). There are promoters and enhancers available for CYP21A2 (Genecards CYP21A2 n.d.). CYP21A2 is mainly located within the endoplasmic reticulum (Genecards CYP21A2 n.d.).

In Zebrafish, *cyp21a2* is also predicted to be involved in the heme-binding activity and glucocorticoid biosynthesis pathway (Eachus et al., 2017 & *ZFIN cyp21a2*, n.d.). It is located on chromosome 16 (*ZFIN cyp21a2*, n.d.). In Zebrafish, the proteins encoded by *cyp21a2* are mainly expressed in the brain and renal system (*ZFIN cyp21a2*, n.d.). Knockout Zebrafish for *cyp21a2* are characterized by an upregulation of hypothalamic-pituitary-interrenal axis and interrenal hyperplasia, reduced cortisol concentrations, and increased concentrations of 17-hydroxyprogesterone and 21-deoxycortisol (Eachus et al., 2017).

Relevant Primary Literature:

Eachus, H., Zaucker, A., Oakes, J.A., Griffin, A., Weger, M., Güran, T., Taylor, A., Harris, A., Greenfield, A., Quanson, J.L., Storbeck, K.H., Cunliffe, V.T., Müller, F., Krone, N. (2017) Genetic disruption of 21-hydroxylase in Zebrafish causes interrenal hyperplasia. Endocrinology. 158(12):4165-4173.

CYP11B2

CYP11B2 is another member of the cytochrome P450 superfamily. Within the STAR biosynthetic pathway, CYP11B2 is involved in the last three steps of the production of aldosterone (OMIM * 124080 n.d.). It is also referenced as ALDOS, CPN2, CYP11B, CYP11BL, CYPXIB2, P-450C18, P450C18, and P450aldo (NCBI CYP11B2 n.d.). In humans, it is located on chromosome 8 and has nine exons (NCBI CYP11B2 n.d.). In humans, mutations of CYP11B2 have been associated with Corticosterone Methyl Oxidase deficiency type 1 and type 2 (Genecards CYP11B2 n.d.). There are promoters and enhancers designed for CYP11B2 (Genecards CYP11B2 n.d.). CYP11B2 is located within the mitochondria in humans and Zebrafish (Genecards CYP11B2 n.d. & *ZFIN cyp11c1*, n.d.). In humans, the protein encoded by CYP11B2 is

mainly expressed in the synovial fluid, adrenal glands, and ovaries (Genecards CYP11B2 n.d.).

In Zebrafish, it was previously referenced as *cyp11b2* and *cyp11b* (*ZFIN cyp11c1*, n.d.). Currently, it is referred to as *cyp11c1* in Zebrafish (*ZFIN cyp11c1*, n.d.). *Cyp11c1* is located on chromosome 16 (*ZFIN cyp11c1*, n.d.). *Cyp11c1* is predicted to be involved in heme-binding activity, iron ion binding activity, oxidoreductase activity, and the cortisol metabolic process (*ZFIN cyp11c1*, n.d.). *Cyp11c1* is mainly expressed in the brain and endocrine system (*ZFIN cyp11c1*, n.d.). *Cyp11c1* encodes for 11 beta-hydroxylase, which is necessary for the biosynthesis of 11-ketotestosterone and cortisol (Zhang et al., 2020). In Zhang et al., they generated a *cyp11c1* knockout Zebrafish (2020). In Zhang et al., they concluded that *cyp11c1* is not required for definite sex differentiation (2020). However, *cyp11c1* is necessary for juvenile ovary-to-testis transition, Leydig cell development, spermatogenesis in males through 11-ketotestosterone, oocyte maturation, and ovulation (Zhang et al., 2020).

Relevant Primary Literature:

Zhang, Q., Ye, D., Wang, H., Wang, Y., Sun, Y. (2020) Zebrafish cyp11c1 knockout reveals the roles of 11-ketotestosterone and cortisol in sexual development and reproduction. Endocrinology. 161(6):.

CYP17A1

CYP17A1 is involved in the production of testosterone and cortisol through the STAR biosynthetic pathway. It is also referenced as CPT7, CYP17, P450C17, and S17AH. CYP17A1 also a member of the cytochrome P450 superfamily (NCBI CYP17A1 n.d.). In humans, it is located on chromosome 10 and has eight exons (NCBI CYP17A1

n.d.). CYP17A1 is most commonly found in the endoplasmic reticulum (Genecards CYP17A1 n.d.).

In humans, the protein encoded by CYP17A1 enables the adrenal glands and gonads to synthesize sex steroids and 17-alpha hydroxylated glucocorticoids (OMIM * 609300 n.d.). CYP17A1 mutations in humans are associated with isolated steroid-17 alpha-hydroxylase deficiency, pseudohermaphroditism, adrenal hyperplasia, cholelithiasis, gallbladder cancer, osteoporosis, and prostate cancer (NCBI CYP17A1 n.d. & *ZFIN cyp17a1*, n.d.). CYP17A1 is mainly found to be expressed in the spinal cord, fetal liver, kidney, adrenal glands, gallbladder, and reproductive organs in humans (Genecards CYP17A1 n.d.). There are 45 types of promoters and enhancers that have been designed for CYP17A1 (Genecards CYP17A1 n.d.). There are 37 drugs and compounds that have been identified to interact with CYP17A1 (Genecards CYP17A1 n.d.)

In zebrafish, *cyp17a1* is located on chromosome 13 (*ZFIN cyp17a1*, n.d.). *Cyp17a1* is involved in female sex determination and progesterone metabolic processes (*ZFIN cyp17a1*, n.d.). It is also mainly expressed in Leydig cells, the brain, and the gonads in Zebrafish (*ZFIN cyp17a1*, n.d.). *Cyp17a1* knockout Zebrafish have been associated with low plasma androgen levels, reduced levels of testosterone and 11-ketotestosterone, and do not display normal male mating behaviors (Shu et al., 2020). Treatment of testosterone or 11-ketotestosterone can restore male mating behaviors in *cyp17a1* knockout Zebrafish (Shu et al., 2020). Overall, *cyp17a1* seems to play a role in zebrafish masculinization and stabilization of androgen levels (Shu et al., 2020).

Relevant Primary Literature:

Shu, T., Zhai, G., Pradhan, A., Olsson, P.E., Yin, Z. (2020) Zebrafish cyp17a1 knockout reveals that androgen-mediated signaling is important for male brain sex differentiation. General and comparative endocrinology. 295:113490

CYP11B1

CYP11B1 is a member of the cytochrome P450 superfamily. It is involved in the production of aldosterone, cortisol, and testosterone in the STAR biosynthetic pathway. Congenital adrenal hyperplasia is a disease associated with CYP11B1 mutations (NCBI CYP11B1, n.d.). This disease is caused by an 11-beta-hydroxylase deficiency from the mutated CYP11B1 (Genecards CYP11B1, n.d.). CYP11B1 has close homology to the CYP11B2 gene (OMIM * 610613, n.d.). There are knockout mice strains with males retaining fertility, but females do not (OMIM * 610613, n.d. & CYP11B1 Gene (Protein Coding), n.d). There are 11 promoters and enhancers designed for CYP11B1 (Genecards CYP11B1, n.d.). CYP11B1 is located within the mitochondria of eukaryotic cells. There are 22 drugs and compounds associated with CYP11B1 (Genecards CYP11B1, n.d.). CYP11B1 is overexpressed within the adrenal gland.

The ortholog of CYP11B1 in Zebrafish is also *cyp11c1* (Genecards CYP11C1, n.d.). As was previously stated for CYP11B2, *cyp11c1* is involved in heme-binding activity, iron ion binding activity, and monooxygenase activity (ZFIN cyp11c1, n.d.). *Cyp11c1* encodes for 11 beta-hydroxylase, which is necessary for the biosynthesis of 11-ketotestosterone and cortisol (Zhang et al., 2020). In Zhang et al., they generated a *cyp11c1* knockout Zebrafish (2020). In Zhang et al., they concluded that *cyp11c1* is not required for definite sex differentiation (2020). However, *cyp11c1* is necessary for

juvenile ovary-to-testis transition, Leydig cell development, spermatogenesis in males through 11-ketotestosterone, oocyte maturation, and ovulation (Zhang et al., 2020).

Relevant Primary Literature:

Zhang, Q., Ye, D., Wang, H., Wang, Y., Sun, Y. (2020) Zebrafish cyp11c1 knockout reveals the roles of 11-ketotestosterone and cortisol in sexual development and reproduction. Endocrinology. 161(6):.

SULT2A1

In humans, SULT2A1 is a member of the sulfotransferase family (Genecards SULT2A1, n.d.). The SULT2A1 gene encodes for a protein that aids in the metabolism of drugs, catalyzes the sulfation of steroids and bile acids in the liver and adrenal glands. It potentially plays a role in excess androgen experienced by women with polycystic ovary syndrome (Genecards SULT2A1, n.d. & OMIM * 125263, n.d.). Within the STAR pathway, SULT2A1 is involved in the testosterone pathway (Bacila, Elder, & Krone, 2019). SULT2A1 also plays a prominent role in maintaining steroid and lipid homeostasis through sulfating steroids and sterols (Genecards SULT2A1, n.d.). In humans, it is allocated on chromosome 19 and is roughly 17 kb with six exons (OMIM * 125263, n.d.). Diseases associated with SULT2A1 are Mixed Epithelial Stromal Tumour and Adrenal Cortical Adenoma (Genecards SULT2A1, n.d.). Orthologs of the SULT2A1 gene for Zebrafish are *sult2st1*, *sult2st2*, and *sult2st3*, with *sult2st1* having the closest similarity (Genecards SULT2A1, n.d.).

Sult2st1 is a member of the sulfotransferase family and is predicted to be involved in sulfation (ZFIN sult2st1, n.d.). While the human ortholog is expressed in the liver and adrenal glands (Genecards SULT2A1, n.d.), the zebrafish ortholog is

expressed in the cardiovascular system, digestive system, and the yolk syncytial layer (ZFIN sult21st1, n.d.). There has not been much direct research and analysis into this specific gene compared to other orthologs in the STAR pathway. However, in Kurogi et al., they found that Zebrafish *Sult2* family members' catalytic properties were different from human SULT2A1, whereas the enzymatic characteristics of zebrafish *Sult3* members, in particular *Sul3st4*, correlated more closely to human SULT2A1 (2017). In Mohammed et al., *sult3st4* exhibited vigorous activity towards dehydroepiandrosterone (DHEA), pregnenolone, and 17β-estradiol (2012). There are currently only five papers published on ZFIN that include *sult3st4, limiting* the available knowledge of the gene.

Relevant Primary Literature:

Kurogi, K., Yoshihama, M., Horton, A., Schiefer, I.T., Krasowski, M.D., Hagey, L.R., Williams, F.E., Sakakibara, Y., Kenmochi, N., Suiko, M., Liu, M.C. (2017) Identification and characterization of 5α-cyprinol-sulfating cytosolic sulfotransferases (Sults) in the Zebrafish (Danio rerio). The Journal of steroid biochemistry and molecular biology. 174:120-127.

Mohammed, Y.I., Kurogi, K., Shaban, A.A., Xu, Z., Liu, M.Y., Williams, F.E., Sakakibara, Y., Suiko, M. Bhuiyan, S., and Liu, M.C. (2012) Identification and characterization of Zebrafish SULT1 ST9, SULT3 ST4, and SULT3 ST5. Aquatic toxicology (Amsterdam, Netherlands). 112-113C:11-18.

HSD11B1

HSD11B1 stands for hydroxysteroid 11-beta dehydrogenase 1 (Genecards HSD11B1, n.d.). It is located on chromosome 1, is roughly 9 kb, and has six exons (Genecards HSD11B1, n.d. & OMIM * 600713, n.d.). HSD11B1 codes for a protein that is a microsomal enzyme involved in catalyzing the conversion of cortisol to cortisone (Genecards HSD11B1, n.d.). High levels of cortisol have been associated with central obesity and insulin resistance in children (Genecards HSD11B1, n.d.). Diseases related

to HSD11B1 mutations are cortisone reductase deficiency and hyperandrogenism (Genecards HSD11B1, n.d.). There are transgenic mice for HSD11B1 (OMIM * 600713, n.d.). There has not been an ortholog of HSD11B1 identified for Zebrafish (Genecards HSD11B1, n.d.). Although there have not been any orthologs identified for HSD11B1 in Zebrafish, there is a zebrafish ortholog (*hsd11b1la*) for the human gene HSD11B1L, which is a paralog for HSD11B1 (ZFIN hsd11b1la, n.d. & Genecards HSD11B1, n.d.). *Hsd11b1la* is located on chromosome 22 and is predicted to be involved in steroid dehydrogenase activity (ZFIN hsd11b1la, n.d.). However, a paralog to 11 beta-hydroxysteroid dehydrogenase type 1 is 11-βHSD3, which Zebrafish have two isoforms of which appear to take part in the conversion of cortisonel to cortisol (Baker, 2010). There have been 19 β-*hsd* genes identified within Zebrafish (Xiao et al., 2020).

Relevant Primary Literature:

Baker, M.E. (2010) Evolution of 11beta-hydroxysteroid dehydrogenase-type 1 and 11beta-hydroxysteroid dehydrogenase type 3. FEBS letters. 584(11):2279-2284.

Xiao, L., Guo, Y., Wang, D., Zhao, M., Hou, X., Li, S., Lin, H., Zhang, Y. (2020) Beta-Hydroxysteroid Dehydrogenase Genes in Orange-Spotted Grouper (*Epinephelus coioides*): Genome-Wide Identification and Expression Analysis During Sex Reversal. Frontiers in genetics. 11:161.

HSD11B2

HSD11B2 stands for Hydroxysteroid 11-Beta Dehydrogenase 2 (Genecards HSD11B2, n.d.). HSD11B2 encodes a protein involved in converting cortisol to cortisone (Genecards HSD11B2, n.d. & OMIM * 614232, n.d.). HSD11B2 is more strictly expressed in the placenta and kidney (OMIM * 614232, n.d.). Diseases associated with HSD11B2 mutation are Apparent Mineralocorticoid Excess and Hypokalemia

(Genecards HSD11B2, n.d.). HSD11B2 is located on chromosome 16, is about 6.2 kb, and has 5 exons (Genecards HSD11B2, n.d. & OMIM * 614232, n.d.).

The ortholog for the HSD11B2 human gene in Zebrafish is *hsd11b2* (Genecards HSD11B2, n.d.). *Hsd11b2* is involved in the negative feedback regulation of cortisol within the brain of Zebrafish (Xiao et al., 2020). *Hsd11b2,* in conjunction with *hsd20b2,* creates a short pathway that rapidly inactivated and excretes cortisol from the system of Zebrafish (Tokarz et al., 2013). *Hsd11b2* also has a role in generating androgen 11-ketotestosterone (Meyer et al., 2012). Human HSD11B2 can be inhibited by dithiocarbamates and organotins (Meyer et al., 2012). When thiram and several organotins were applied to Zebrafish *hsd11b2*, there was weak inhibition by thiram and no inhibition by organotins (Meyer et al., 2012). There are currently three mutant Zebrafish for the *hsd11b2* gene, with one having a premature stop and the other two with their consequences unknown (ZFIN Hsd11b2, n.d.).

Relevant Primary Literature:

Xiao, L., Guo, Y., Wang, D., Zhao, M., Hou, X., Li, S., Lin, H., Zhang, Y. (2020) Beta-Hydroxysteroid Dehydrogenase Genes in Orange-Spotted Grouper (*Epinephelus coioides*): Genome-Wide Identification and Expression Analysis During Sex Reversal. Frontiers in genetics. 11:161.

Meyer, A., Strajhar, P., Murer, C., Da Cunha, T., and Odermatt, A. (2012) Species-specific differences in the inhibition of human and zebrafish 11beta-hydroxysteroid dehydrogenase 2 by thiram and organotins. Toxicology. 301(1-3):72-78.

Tokarz, J., Norton, W., Möller, G., Hrabé de Angelis, M., and Adamski, J. (2013) Zebrafish 20beta-hydroxysteroid dehydrogenase type 2 is important for glucocorticoid catabolism in stress response. PLoS One. 8(1):e54851.

HSD17B1

HSD17B1 stands for hydroxysteroid 17-beta dehydrogenase 1 (Genecards HSD17B1, n.d.). The protein encoded by HSD17B1 is responsible for the interconversion of estrone and estradiol as well as the interconversion of androstenedione and testosterone (OMIM * 109684, n.d.). HSD17B1 is located in the ovaries, testes, placenta, and various peripheral tissues (uterus, breast, prostate, and fat) (OMIM *109684, n.d.). Diseases associated with HSD17B1 mutations include ovarian disease and hypothalamic obesity (Genecards HSD17B1, n.d.). There are currently three mouse knockouts for HSD17B1 (Genecards HSD17B1, n.d.). A paralog of this gene is RDH16 (Genecards HSD17B1, n.d.). The ortholog for HSD17B1 in Zebrafish is *hsd17b1* (Genecards HSD17B1, n.d.).

Within Zebrafish, *hsd17b1* encodes for a protein involved in the androgen metabolic and estrogen biosynthetic processes (ZFIN hsd17b1, n.d.). There is currently one transgenic zebrafish mutant for *hsd17b1,* but the localization and consequence are unknown (ZFIN hsd17b1, n.d.). *Copper impacts Hsd17b1* within surface water, negatively impacting steroid hormone levels (Wang et al., 2019). The loss of androgen receptor within Zebrafish results in deregulated *hsd17b1* gene regulation within testes and ovaries (Zhang et al., 2018). There does not appear to be extensive research focused on *hsd17b1* exclusively.

Relevant Primary Literature:

Cao, J., Wang, G., Wang, T., Chen, J., Wenjing, G., Wu, P., He, X., Xie, L. (2019) Copper caused reproductive endocrine disruption in Zebrafish (Danio rerio). Aquatic toxicology (Amsterdam, Netherlands). 211:124-136.

Yu, G., Zhang, D., Liu, W., Wang, J., Liu, X., Zhou, C., Gui, J., Xiao, W. (2018) Zebrafish androgen

receptor is required for spermatogenesis and maintenance of ovarian function. Oncotarget. 9:24320-24334.

Ethical Reflection on CRISPR/Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) bacterial defense system was discovered by Spanish microbiologist Francisco Mojica in 1993 (Baylis, 2019 & Eissenberg, 2021). The CRISPR-associated component is commonly referred to as Cas9 since it is a protein from the bacterium *Streptococcus pyogenes* (Eissenberg, 2021). In 2012, American biochemist Jennifer Doudna and French microbiologist Emmanuelle Charpentier applied CRISPR/Cas9 to genome engineering (Baylis, 2019). Using CRISPR/Cas9, the genome can be altered through the insertion or deletion of nitrogenous bases (Adenine, Cytosine, Thymine, and Guanine) (Baylis, 2019). CRISPR/Cas9 genome editing technology holds the potential to broadly impact and transform the methods related to human health, the cultivation and management of livestock and agriculture, and pest species management (Eissenberg, 2021).

CRISPR/Cas9 can be utilized to edit somatic cells and germline cells (Baylis, 2019). Genomic editing for somatic cells and germline cells includes modifying a gene either by targeted inactivation or targeted replacement (Eissenberg, 2021). Somatic cells differ from germline cells in that they are nonreproductive cells that only impact the patient (Brokowski, 2018). Examples of nonreproductive somatic cells include cardiomyocytes, monocytes, and osteoblasts (Brokowski, 2018). Somatic cell CRISPR/Cas9 editing can impact the existing patient through treatment, prevention of

premature death, or relief of suffering (Eissenberg, 2021). The overall goal of somatic genome editing is to create an impact on the existing patient (Baylis, 2019 & Brokowski, 2018).

In contrast to somatic genome editing, germline genome editing aims to generate a modified human that can transfer the genomic change to future generations (Baylis, 2019 & Brokowski, 2018). Germline genome editing, also referred to as heritable genome editing, involves genetic modification of reproductive cells and early-stage embryos, which ultimately impacts offspring and future generations (Baylis, 2019 & Brokowski, 2018). Germline genome editing can influence future generations due to engineering gametes or cleavage-stage cells which are totipotent (Eissenberg, 2021). Totipotent cells ultimately give rise to all somatic cell lineages and germline cells in a developing individual (Eissenberg, 2021). This distinction between somatic genome editing and germline genome editing has led to the use of CRISPR/Cas9 for germline genome editing to be referred to as "reproductive CRISPR" or "rCRISPR" for short (Eissenberg, 2021).

The high-speed development and usage of CRISPR/Cas9 genome editing technology have caught the world by storm, especially when it comes to predicting the future of humanity after the birth of CRISPR/Cas9 genetically edited twin girls in China by Dr. He (Kleiderman & Ogbogu, 2019 & Smith, 2020). Due to the rapid growth of CRISPR/Cas9 technology and advancements in human clinical trials, it is critical that the potential risks and benefits of CRISPR/Cas9 genome editing be thoroughly evaluated before continued practice on humans (Brokowski, 2018). The current CRISPR/Cas9 genomic editing technology challenges include the possibility of an

unpredictable genetic alteration that could result in unintended secondary modifications or effects (Eissenberg, 2021). The gravity of the potential influence that CRISPR/Cas9 holds on the future of humanity has led some educators to create courses centered around CRISPR/Cas9 to spread awareness of the technology and the potential applications to society (Smith, 2020).

The rapid growth of CRISPR/Cas9 and the possible applications to humanity through the editing of somatic and germline cells have captivated many individuals and spurred the creativity of many. One notable individual who has fully embraced CRISPR's possible uses is Josiah Zayner (Baylis, 2019). Josiah Zayner is the founder of The ODIN, a company built on the belief of participatory science while also democratizing science (Baylis, 2019). Zayner stands apart within the scientific community, most notably for his public displays of self-experimentation (Baylis, 2019). In 2017, in San Francisco at a synthetic biology industry meeting (SynBioBeta), Zayner injected himself in the arm with CRISPR/Cas9 with the hopes of deleting the myostatin gene that regulates muscle growth (Baylis, 2019). The public display of self-injection by Zayner led to the development and sale of CRISPR "treatments" by the biohacking company Ascendance Biomedical (Baylis 2019). This public display was shortly followed by the U.S. Food and Drug Administration (FDA) issuing a warning against the use of self-administered CRISPR "treatments" and making them illegal to sell to the public (Baylis, 2019).

CRISPR/Cas9 is predated by genome editing technology referred to as zinc finger nucleases and the transcription activator-like effector nucleases (TALENs) (Eissenberg, 2021). Both the zinc finger nucleases and TALEN accomplished genomic

editing by delivering a DNA-cleaving endonuclease to a specific genome target recognized by a particular nucleotide sequence (Eissenberg, 2021). Zinc finger nucleases, along with TALEN, result in a double-stranded break at the target site of the DNA, which can result in mutations that inactivate the gene function (Eissenberg, 2021). CRISPR/Cas9 is different from zinc finger nucleases, and TALEN in that CRISPR/Cas9 uses RNA to target a bound protein endonuclease instead of DNA-binding peptide sequences to target specificity (Eissenberg, 2021).

CRISPR/Cas9 effectively edits the genome by cleaving both strands of DNA at the target site through the Cas9 endonuclease bound to the CRISPR guide RNA (Eissenberg, 2021). The double-stranded break results in the cells repairing the crack through non-homologous end-joining or homologous recombination (Eissenberg, 2021). Non-homologous end-joining is a default response of the cell to fix a double-stranded break in DNA (Eissenberg, 2021). It is also known for inducing errors which often results in deletions or insertions of nucleotides, creating a mutation (Eissenberg, 2021). Homologous recombination can begin if an intact homologous DNA molecule is available, resulting in a different DNA sequence being inserted in the repair site (Eissenberg, 2021).

The possible use of CRISPR/Cas9 for genetic therapeutic purposes also was paved by gene transfer experiments and clinical practices in the 1980s and 1990s, better known as gene therapy (Baylis, 2019 & Brokowski, 2018). Gene therapy involves genetically engineering organisms for either investigational or therapeutic practices (Brokowski, 2018). An early gene transfer clinical experiment for ornithine transcarbamylase in 1999 was accompanied by the death of eighteen-year-old Jesse

Gelsinger (Baylis, 2019 & Brokowski, 2018 & Eissenberg, 2021). Baylis emphasized that the associated risk of death through possible CRISPR/Cas9 clinical trials, somatic or germline, should be assumed as risky as early gene transfer research (2019). However, there is support for somatic cell CRISPR/Cas9 editing within humans due to the argument that it is equivalent ethically to transgenic therapies (Eissenberg, 2021).

Based on gene therapy, it can also be assumed that CRISPR/Cas9 genome editing therapy would be relatively exclusive and expensive, as was the first licensed gene therapy drug Glybera (Baylis, 2019). Glybera was a gene therapy drug that was meant to correct lipoprotein lipase deficiency (Baylis, 2019). When Glybera aired on the market, it was sold at one million U.S. dollars for one single dose (Baylis, 2019). Luxturna is another gene therapy drug that treats biallelic RPE65 mutation-associated retinal dystrophy (Baylis, 2019). Luxturna was released to the market in 2018 for $850,000 in the U.S. (Baylis, 2019).

The grandiose prices associated with gene therapy drugs may only be a fraction of the potential cost associated with CRISPR/Cas9 genome editing therapy, which poses a limiting factor on how obtainable it is for patients within the United States. The United States does not practice universal healthcare but is dependent on citizens' insurance status or ability to pay out of pocket (Baylis, 2019). The likelihood of the average U.S. citizen's ability to afford possible future CRISPR/Cas9 genome editing therapy with or without insurance is slim due to the estimated high cost of the technology (Baylis, 2019).

An example of widely denounced CRISPR/Cas9 applied genome editing is the clinical trial Dr. Jiankui He conducted, which resulted in the birth of germline-edited twin

girls in 2018 (Kleiderman & Ogbogu, 2019). While Dr. He's research was able to accomplish the delivery of CRISPR/Cas9 germline edited twin girls, it appears that his research violated both Chinese regulations and internationally accepts research and bioethical standards, which puts into question the enforcement of Chinese regulations (Kleiderman & Ogbogu, 2019). The 2016 Chinese *Ethical Review Guidelines on Biomedical Research Involving Human Subjects* addresses ethical matters such as informed consent, voluntary participation in research, risk-benefit assessment, compensation for participants, and ethics approval by provincial or national committees before the start of the study (Kleiderman & Ogbogu, 2019). China also has two specific sets of guidelines centered on the ethical conduct of human embryo research (Kleiderman & Ogbogu, 2019). These two guidelines include the *Ethics Guiding Principles for hESC Research ("Guiding Principles")* and the *Technical Norms on Human Assisted Reproductive Technologies ("Technical Norms")* (Kleiderman & Ogbogu, 2019).

The *"Guiding Principles"* clearly prohibits using genetically modified/manipulated human gametes, zygotes, and embryos for reproduction (Kleiderman & Ogbogu, 2019). It is clear that Dr. He violated the *"Guiding Principles"* by the birth of the CRISPR/Cas9 genetically modified twin girls. Dr. He also violated Chinese guidelines through lack of transparency in the design and methods of the research and clinical trial, lack of a formal ethics review, and the lack of an approved risk-benefit assessment (Kleiderman & Ogbogu, 2019). Dr. He's research highlights the issues surrounding the enforcement and implementation of research and clinical ethics guidelines with China (Kleiderman & Ogbogu, 2019). Dr. He's research also created an urge for the development of globally

applicable bioethics guidelines concerning research using CRISPR/Cas9 technology. However, the development of globally applicable bioethics guidelines has to consider cultural variation, diversity in values and principles, which creates difficulty in creating such policies (Kleiderman & Ogbogu, 2019).

To further discuss the possible implications of CRISPR/Cas9 genome editing within human trials, seven standard requirements must be met to consider research involving human participants as ethically sound (Kleiderman & Ogbogu, 2019). These seven ethical requirements include social or scientific value, scientific validity, fair subject selection (protecting vulnerable or stigmatized human participants), favorable risk-benefit ratio, independent scientific and ethics review, informed consent, and respect for subjects (Kleiderman & Ogbogu, 2019). These seven standard ethical requirements for research involving human participants are highly considered within scientific groups concerning their stance on CRISPR/Cas9 genome editing technology.

Statements regarding the use of CRISPR/Cas9 genome editing within humans range between scientific groups and countries (Brokowski, 2018). Two scientific groups from the Netherlands, the Netherland Commission on Genetic Modification and the Health Council of the Netherlands, issued a joint report in 2017 that if safe therapeutic applications of germline modification were possible that it could be a moral obligation of healthcare providers to offer it to the public (Brokowski, 2018). Similar to the Netherland Commission on Genetic Modification and the Health Council of the Netherlands, the United Kingdoms Academy of Medical Sciences also favors implementing CRISPR/Cas9 technology to public consumption once the technology has a solid evidence base, matches societal values, and has public support (Brokowski, 2018). This

statement is in stark contrast with the statement released by the National Institute of Health (NIH) Director Francis Collins, stating that the lack of compelling medical applications, unquantifiable safety issues, and the ethical issues that impact future generations do not present a case for the use of CRISPR/Cas9 within humans (Brokowski, 2018). The statement issued by the International Summit on Human Gene Editing in 2015 on the usage of CRISPR/Cas9 editing technology within humans fell between the two ends of the spectrum. The overall conclusion from the International Summit on Human Gene Editing was that it would be irresponsible to implement CRISPR/Cas9 technology until the safety concerns are more thoroughly understood (Brokowski, 2018).

Safety concerns for using CRISPR/Cas9 on humans for both somatic and germline genome editing include the possibility of off-target modifications, uncertain efficiency, and unintended consequences of genomic modification that could increase safety risks (Brokowski, 2018). Off-target alterations to the genome include unwanted and unpredictable genetic alterations (Baylis, 2019). Inaccurate CRISPR/Cas9 editing could result in simple off-target modifications that include the change of a single nitrogenous base (point mutation), insertion of a nitrogenous base(s), or unwanted deletion of a nitrogenous base(s) (Baylis, 2019 & Brokowski, 2018). It has also been observed that repeated cycles of somatic genome editing are associated with the risk factor of triggering an immune response to the genomic editing technology (Eissenberg, 2021).

Another concern of the possible impact of CRISPR/Cas9 genomic modification on society includes the possible reinforcement and enlargement of prejudices and

inequality within society (Brokowski, 2018). Regarding using CRISPR/Cas9 genetic editing technology for specifically enhancement procedures, some individuals have opposition to using CRISPR/Cas9 genetic modification for uses outside of medical concerns due to a fear of fueling prejudices and inequality (Baylis, 2019). Medical enhancement procedures differ from medical treatments in that the goal is to create an unnecessary improvement or change in a patient without a genuine medical deficiency, such as improved athletic performance (Baylis, 2019). The ability for parents to choose genetically desirable enhancements for their children could result in more significant prejudices against undesirable traits and individuals that possess them. Interestingly, suppose limitations were placed on the use of CRISPR/Cas9 technology to only a setlist of medical concerns. In that case, it could potentially be argued that it is an infringement on the 1st Amendment freedom of speech (Brokowski, 2018). It can also be argued that medical treatments are equivalent to enhancements since they both aim at the improvement of the patient through the correction of an actual or perceived deficiency (Baylis, 2019).

Overall, the research and development of CRISPR/Cas9 germline editing technology (rCRISPR) for use within society is not a morally urgent issue since there are already safer, simpler, and cheaper alternatives available to individuals (Baylis, 2019 & Eissenberg, 2021). Alternatives to rCRISPR are pre-implantation genetic diagnosis, sperm or egg donor, foster parenting, and adoption (Baylis, 2019 & Eissenberg, 2021). Pre-implantation genetic diagnosis involves the screening for embryos free of a specific gene during *in vitro* fertilization (IVF) (Eissenberg, 2021). It can also be argued that the vast amount of time, talent, and finances required to

establish the availability of rCRISPR to the public would not be met be with the appropriate level of consumption to match the resources expended (Baylis, 2019).

However, there is debate over whether or not the desire for a genetically healthy and genetically related child is a "compelling medical need" (Baylis, 2019 & Eissenberg, 2021). The common arguments supporting rCRISPR for a genetically healthy/related child are parent-child physical resemblance, family resemblance, psychological similarity, for love, for a genetic connection, to be a procreator, and to experience pregnancy (Baylis, 2019). American philosopher Tina Rulli critically examined the common arguments used in support of rCRISPR and concluded that each view could be classified as "wanting" instead of needing (Baylis, 2019). Another idea for the use of rCRISPR within society is that over a span of generations, it would reduce the incidence of genetic disease in individuals, which would enhance the gene pool while also increasing the availability of finances to stimulate other areas of the economy besides healthcare (Baylis, 2019).

Although the expansive growth within the application of CRISPR/Cas9 genome editing technology is a noteworthy accomplishment within the history of humanity, it is essential to remember the associated risk factors that have not been solved before pushing for the availability of public consumption (Eissenberg, 2021). While CRISPR/Cas9 holds hope in positively influencing the future treatment and management of genetic diseases in somatic and germline cells, there are currently alternatives available to the public, such as gene therapy treatments or IVF (Baylis, 2019 & Eissenberg, 2021). Instead of further encouragement of putting more resources towards the research and development of CRISPR/Cas9, it is essential to be reminded

of more pressing problems in the world to put resources towards, such as overpopulation, climate change, water scarcity, environmental degradation, and food insecurity (Baylis, 2019). While eliminating or diminishing the presence of genetic diseases through CRISPR/Cas9 within future generations would be magnificent, it is crucial that the Earth's environmental integrity is maintained and repaired for future generations' insurance, health, and sustainability.

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