# Investigating the impacts of combination oral contraceptive pills on the connective tissues of female patients with Ehlers Danlos Syndromes: A Proposal

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**Abstract**

Ehlers Danlos Syndromes (EDS) are a class of 13 genetically similar inherited connective tissue disorders, typically categorized by joint hypermobility and connective tissue abnormalities. Due to the resulting connective tissue laxity and issues in collagen synthesis, female EDS patients experience higher rates of gynaecological impact such as dysmenorrhea, hypermenorrhea, and menorrhagia. Healthcare providers (HCPs) are increasingly prescribing combination estrogen-progesterone monophasic oral contraceptive pills (OCPs) as the primary method to treat these conditions. While previous studies have demonstrated interactions between sex hormones and collagen, the impacts of increased estrogen and progesterone levels on those with connective tissue disorders, which typically lead to prescription of OCPs, has yet to be studied. This has led to a knowledge gap on how increased hormone levels interact with the pathogenic collagen produced in EDS. To fill this gap, using CRISPR-Cas9 genome editing, a mouse model can be created to represent classical EDS (cEDS) using known mutations. Using this model, it is then possible to directly analyze the impacts of fourth and second generation combination OCPs on connective tissue in those with cEDS. This can be done using translationally relevant doses in mice of both ethinyl estradiol and drospirenone to mimic fourth generation OCPs, and ethinyl estradiol and levonorgestrel to mimic second generation OCPs. These models can be used to determine if there is any interaction between estrogen, progesterone, and disordered collagen, and to see if these interactions lead to better or worse outcomes long-term in cEDS, and other connective tissue disorder, patients.

**Key Words**

Ehlers-Danlos Syndrome, birth control, connective tissue, collagen, estrogen, progesterone

**Scientific Background**

Ehlers-Danlos Syndromes (EDS) are a group of 13 complex, autosomal connective tissue disorders, frequently displaying joint hypermobility, skin hyperextensibility and fragility, as well as joint dislocations and subluxations [1]. Some classes of the disorder are more severe, while other types, such as the classical subtype (cEDS), present with skin fragility, irregular scarring, and joint hypermobility [1]. All but one class of EDS has genetic markers associated with them [1].

The majority of EDS cases are caused by mutations in genes responsible for collagen synthesis [1]. cEDS has been linked primarily to a terminal deletion of exons 12-57 within the COL5A1 gene, at 9q34.3 [2]. Though many phenotypically congruent mice lines have been developed [3], the ($Col5a1^{+/-}$) line, heterozygous for a knockout in the Col5a1 gene, is the most promising line for cEDS research, with affected mice showing a 50% decrease in functional type V collagen, similar to human cEDS patients [3].

Recent studies have shown that women with EDS show a marked increase in gynaecological impacts, with 92.5% of female EDS patients experiencing dysmenorrhea [4], and 32.9% experiencing menorrhagia [4]. The main symptom management method for gynaecological conditions is the prescription of the combination oral contraceptive pill, or OCPs [5]. In the US alone, around 14% of women use OCPs [5], with 14% of OCP users stating they do not take them for contraceptive reasons [6]. These pills are typically ethinyl estradiol (EE), as well as one of the four classes of progestins [5].

Combination OCPs relieve symptoms of various gynaecological issues by using progestins to suppress ovulation, and estrogens to regulate bleeding [6]. Previous studies have demonstrated how increases in estrogen are linked to an increase in collagen synthesis [7, 8]. They have also demonstrated how high doses of progesterone are linked to a decrease in collagen synthesis and an increase in collagen degradation, notably in the cervix [9]. However, in many EDS patients, collagen is often insufficient or misformed [1]. As EDS patients are often prescribed birth control for gynaecological issues, it is important to investigate if the increase in sex hormones causes any effect on damaged connective tissues.

**Research Question and Hypothesis**

The goal of this research is to determine if there is any impact of the increased levels of estrogen and progesterone caused by combination OCPs on the connective tissue of patients with Ehlers Danlos Syndrome.

If combination OCPs prove to interact with the damaged connective tissue of female EDS patients, then it may fill a gap in our knowledge on both EDS and OCPs, and may demonstrate the need for the development of other treatments for this class of disorders.

**Rationale**

Females with EDS experience higher rates of gynaecological issues [4]. Due to the fact that combination OCPs are seen as their primary treatment method [4], they likely have a high rate of prescription among EDS patients. Recent research has identified interactions between estrogen, progesterone, and collagen, the main protein affected in EDS [7-9]. Progesterone, having already been found to potentially destabilize healthy collagen [9], may have more negative effects on EDS than previously known. If so, female EDS patients may be forced to decide between debilitating gynaecological symptoms, or treatment with OCPs that cause aggravated symptoms in other regions of the body.

**Methodology**

Using CRISPR/Cas9 genome editing, a ($Col5a1^{+/-}$) line of mice that mimics the phenotype of cEDS [3] can be developed. After 60 days of growth and development to fully reach sexual maturity, each line will be split into either a control group (Group 0A), a fourth generation OCP treatment group (Group 1A), or a second generation OCP treatment group (Group 2A).

Group 0A will receive a 250uL placebo dose of just 10% sucrose water [10] once per day, daily, for 260 days. Group 1A will be given a 250uL dose of 10% sucrose, 0.01875ug/20g mouse of EE, and 3.75ug/20g mouse of drospirenone (DRSP) daily for 260 days to mimic a fourth-generation OCP. Group 2A will be given a 250uL dose of 10% sucrose, 0.01875ug/20g mouse of EE, and 0.75ug/20g mouse of levonorgestrel (LVNG) daily for 260 days to mimic a second-generation OCP. All of these doses are based on prior publications which found success in administering relevant doses of birth control to mice [10]. These same trials will also be repeated with an inbred control line of C57BL/6 mice, with the same treatments. These groups will be called Group 0B, Group 1B, and Group 2B respectively. The treatment solution should be delivered at the same time daily, and mice should be monitored after solution is given to ensure it is all consumed.

**Table 1. Experimental Design, Controls vs. Mutants vs. Exposure Status**

| Mouse Line | Birth Control Exposure Status | | |
|---|---|---|---|
| [redacted] | 250uL: 10% sucrose (Control) | 250uL: 10% sucrose 0.01875ug/20g EE 3.75ug/20g DRSP (4th Gen. OCP) | 250uL: 10% sucrose 0.01875ug/20g EE 0.76ug/20g LVNG (2nd Gen. OCP) |
| $Col5a1^{+/-}$ (Mutant) | Mutated / Control (positive control 0A) | Mutated / 4th Gen (Group 1A) | Mutated / 2nd Gen (Group 2A) |
| C57BL/6 (Control) | Control / Control (negative control, 0B) | Control / 4th Gen (Group 1B) | Control / 2nd Gen (Group 2B) |

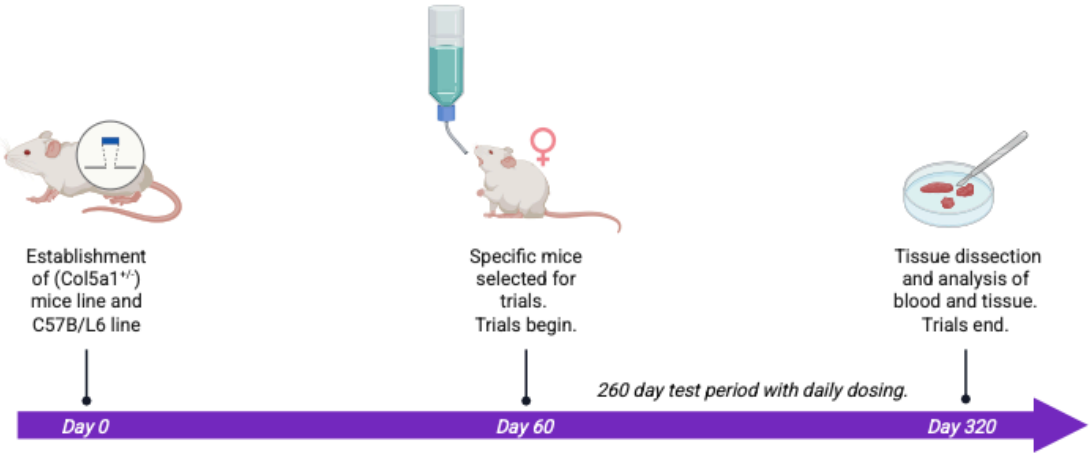


**Figure 1. Experimental timeline.** Female mice averaging around 20g will be selected for trials. After the test period concludes, detailed analysis will be done. Created in https://BioRender.com

After a 260 day test period with daily dosing, the mice from each group will be dissected. Their connective tissue will be analyzed, and then compared with their respective controls to determine any differences in molecular collagen makeup, connective tissue structure, and tissue fragility.

Blood analysis will be done before dissection. Any obvious behavioral changes not seen in the control line shall be noted throughout the wait period.

**Conclusion**

EDS patients already experience a multitude of frustrating symptoms that often impact quality of life. In female patients, gynaecological comorbidities are common [4], and are treated with combination OCPs [6]. However, we currently do not know if the hormones within combination OCPs interact differently with healthy collagen compared to the disordered collagen in EDS patients. There is a chance that women with EDS who are prescribed OCPs for gynaecological issues will experience more debilitating symptoms in other regions of the body due to the increase in hormone levels, leading to a critical problem where a medication does more harm than good. Investigations into the interactions of estrogen, progesterone, and the disordered type V collagen within cEDS patients can help open the door to more research of this type, and may reveal a dire need for new and improved treatments for gynaecological disorders, not only for EDS patients, but for all those who need it.

**Definitions Sheet**

- Ehlers Danlos Syndrome: A class of connective tissue disorders typically caused by mutations in various collagen genes, or genes linked to collagen-related ECM proteins. Linked to tissue fragility, joint hypermobility, chronic pain, and generalized musculoskeletal instability. [1]
    - Classical Ehlers Danlos Syndrome: A subset of EDS caused by mutations within the COL5A1 or COL5A2 genes. Believed to be the second most common form of EDS, behind hypermobile EDS. [1]
- Estrogen: The main class of female sex hormones, produced primarily within the ovaries. Responsible for ovarian follicle growth, vaginal wall thickness, mucous secretions, and formation of breast tissue.
    - Estradiol: Most common form of estrogen produced by the female body at reproductive age. Contributes to sexual development.
        - Ethinyl estradiol (EE): A synthetic form of estrogen, most commonly prescribed form of estrogen in OCPs. [11]
- Dysmenorrhea: Abnormal pain during the menstrual cycle, typically at menstruation. [4]
- Exons: Coding regions found within an mRNA strand. Spliced together to create different genes.
- Genetic markers: Specific mutational signatures found in specific genetic diseases that can be used to easily identify and diagnose patients with the condition.
- Inbred strain: A line of mice which are genetically identical throughout the genome due to generational inbreeding. A lack of genetic differences within the strain means they are free from individual genetic influences that may sway research results. [12]
    - C57BL/G mouse line: Most used inbred strain in research. [12]
- Menorrhagia: Excessive or heavy bleeding during menstruation. [4]
- Monophasic combination oral contraceptive pills (OCPs): Class of combination birth control which uses the same doses of estrogen and progestins in each active pill [5]
- Phenotypically congruent: A phenotype in a genetically engineered animal that mimics the human form of the condition.

- Progesterone: Secondary class of female sex hormones. Produced by the corpus luteum after ovulation. Responsible for the menstrual cycle and pregnancy. Four main classes. [5]
    - Progestin: Artificially developed progesterones used in combination and progesterone-only OCPs [13]
        - Drospinerone (DRSP): A class IV progestin. [5]
        - Levonorgestel: LVNG): A class II progestin. [5]
- Subluxation: A partial joint dislocation where joint surfaces are still in contact, but the joint is not in proper position.
- Terminal deletion: A chromosomal abnormality with a loss of genetic material at the ends of the chromosome
- Translationally relevant dose: A dosage of a medication which causes the same relative impacts on the studied animal compared to humans. Not always directly correlated to body weight. [14]
- Type V collagen: A regulatory, fibril-forming collagen. Deficient in cEDS [1]. Binds to ECM molecules and other collagen proteins, promoting structural integrity, cellular behaviour, and regulation of macromolecules within the ECM. Overexpression is linked to cancer development [15]. In humans, encoded primarily by the COL5A1 and COL5A2 genes [1].

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