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CLINICAL, PSYCHOLOGICAL, EPIGENETIC, AND MEDICO-LEGAL ASPECTS RELATED TO THE DEVELOPMENT OF POSTTRAUMATIC STRESS DISORDER IN VIOLENCE AGAINST WOMEN

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INTRODUCTION

Violence against women is a pervasive and multifaceted issue that transcends national borders, socio-economic strata, and cultural contexts. It remains a social problem that not only infringes upon the fundamental rights of women but also has long-lasting, detrimental impacts on their physical, psychological, and emotional well-being. Globally, this phenomenon affects millions of women, manifesting in forms such as physical assault, sexual abuse, psychological manipulation, and economic deprivation. In Italy alone, data from the Italian National Institute of Statistics (ISTAT) reveal that 31.5% of women between the ages of 16 and 70 have experienced some form of physical or sexual violence during their lives. These staggering statistics point to the ubiquity and gravity of the problem, signaling the need for comprehensive responses from both public institutions and the scientific community.

In recent years, the rise in IPV cases, particularly during the COVID-19 pandemic, has further highlighted the urgent need for systematic approaches to address this issue. The pandemic not only amplified cases of violence but also brought to light what has been termed a "shadow pandemic," a silent and pervasive crisis that remains underreported and often invisible to formal health and social services. This situation has further exacerbated the psychological and physical suffering of women who endure violence in confined domestic spaces, and it underscores the need for more understanding and intervention.

The damage inflicted by gender-based violence is not limited to visible physical injuries but extends to deep, often irreversible changes in the biological and psychological landscapes of the survivors. While immediate physical effects such as bruises, fractures, or wounds are more easily detectable and addressed through medical intervention, the long-term effects of violence on psychophysical health are more insidious and often escape clinical detection. The psychological scars, such as those linked to post-traumatic stress disorder (PTSD), depression, anxiety, and other mental health disorders, are well documented. However, a growing body of evidence points to a more profound, cellular-level impact, wherein the trauma of violence leaves lasting marks on a victim's genome.

This recognition of the deeper biological consequences of violence against women sets the stage for the Epigenetics for Women project (EpiWE), a pioneering initiative dedicated to uncovering the epigenetic changes induced by trauma and violence. The roots of this research can be traced back to 2015, with the launch of the EPIgenetic Repellere Vulnera Ad Mulierem et Puerum project (EpiREVAMP), which was among the first large-scale efforts to explore how violence—an extreme environmental stressor—can affect the genomic health of both women and children. The EpiWE project, which builds on the foundational work of EpiREVAMP, seeks to delve into the role of epigenetics in understanding the long-term health consequences of violence and trauma.

Epigenetics, a relatively new field within the biological sciences, offers a revolutionary lens through which to examine the lasting effects

of violence. Unlike genetic mutations, which alter the DNA sequence itself, epigenetic modifications involve changes in gene expression that do not affect the underlying DNA code but can nonetheless have profound impacts on health. These changes, triggered by environmental factors such as trauma, act as a form of biological memory, recording the body's response to external stimuli. In the context of violence against women, this means that traumatic events can leave lasting imprints on the genome, which may predispose survivors to developing chronic health conditions such as cardiovascular diseases, autoimmune disorders, and cancers later in life.

The EpiWE project, led by the Italian National Institute of Health in collaboration with the University of Milan and other medical institutions, aims to identify and study these epigenetic modifications in women who have survived violence.

This thesis presents the pilot study of the project that has already produced interesting results, identifying changes in the expression of three specific genes in women who have experienced trauma. This represents a first step in a larger research agenda that seeks to expand the sample size, incorporate additional research centers, and establish a dedicated biobank. The biobank will facilitate the collection and analysis of biological samples over time, providing invaluable insights into how the epigenome of survivors evolves in response to trauma and its potential role in the onset of disease.

This focus on the epigenetic "scars" left by violence is particularly significant in the context of understanding not only PTSD but also other chronic, non-communicable diseases that may develop as a result of

prolonged exposure to violence. For example, epigenetic markers could help to explain the pathways through which trauma contributes to the development of cardiovascular conditions, metabolic disorders, and even cancer. This research holds promise not only for advancing our understanding of the biological impacts of violence but also for informing the development of precision medicine approaches tailored to the unique needs of survivors. By identifying specific epigenetic markers, it may become possible to design individualized interventions that target these modifications and mitigate their impact on long-term health outcomes.

In addition the implications of this research extend far beyond the individual survivor, as the effects of violence often ripple through entire families and communities. Children who witness ill-treatment are particularly vulnerable to developing emotional and behavioral disorders, and the intergenerational transmission of trauma has been well documented in psychological literature. However, the biological transmission of trauma, potentially mediated by epigenetic changes, remains a relatively underexplored area of study. By deepening our understanding of these mechanisms, the EpiWE project could contribute to the development of strategies aimed at breaking the cycle of violence and its transmission across generations.

One of the key strengths of the EpiWE project is its emphasis on multidisciplinary collaboration. Violence against women is a complex issue that requires input from a wide range of sectors, including healthcare, social services, legal frameworks, and research institutions. The integration of epigenetic research with clinical practice and social

policy represents a holistic approach to addressing the needs of survivors. In addition to its scientific goals, the EpiWE project highlights the importance of creating comprehensive care pathways that provide survivors with the medical, psychological, and social support necessary for recovery. This integrated model recognizes that violence is not only an individual experience but also a social issue with far-reaching consequences.

In addition to advancing scientific knowledge, the EpiWE project also plays a crucial role in advocating for better policy frameworks and interventions. The establishment of a national database on gender-based violence in 2019, through the collaboration between ISTAT and the Ministry of Health, marks a significant step forward in the effort to systematize data collection and analysis on this issue. However, much work remains to be done to ensure that this data can be used effectively to inform prevention strategies and improve the health outcomes of survivors. Developing a linkage system that allows for the integration of individual health records across various databases could help to identify risk profiles and facilitate earlier interventions, ultimately improving the prospects for recovery and reducing the burden on healthcare systems.

In conclusion, violence against women is not merely a violation of human rights; it is a public health crisis that demands a comprehensive, multifaceted response. The EpiWE project, through its innovative focus on the epigenetic impacts of trauma, offers a novel approach to understanding and addressing the long-term health consequences of violence. Through its emphasis on precision medicine,

multidisciplinary collaboration, and the integration of scientific research with clinical practice, EpiWE has the potential to revolutionize the way we approach the prevention and treatment of violence-related diseases, providing hope for a future where survivors are supported not only in their immediate recovery but throughout their lives.

Chapter I
THE PHENOMENON OF VIOLENCE AGAINST WOMEN: EPIDEMIOLOGICAL, LEGISLATIVE, AND CLINICAL PERSPECTIVES.

Contents: 1.1 Conceptual definitions of violence against women. 1.2 Prevalence and patterns of violence against women. 1.3 Italian legal framework addressing violence against women. 1.4 Clinical presentations and health impacts of violence against women.

1.1. Conceptual definitions of violence against women.

The United Nations defines violence against women as any act of gender-based violence that results in, or is likely to result in, physical, sexual, or mental harm or suffering, including threats of such acts, coercion, or arbitrary deprivation of liberty, whether occurring in public or private settings[1]. This definition encompasses a broad range of violent behaviors that undermine women's well-being.

Violence against women is often categorized into two primary domains:

Intimate Partner Violence (IPV): commonly referred to as domestic violence, IPV involves a pattern of abusive behavior by a current or former intimate partner that causes physical, sexual, or psychological

[1] World Health Organization (WHO), *Violence against women - Fact sheet*, 2024, https://www.who.int/news-room/fact-sheets/detail/violence-against-women (20/10/2024).

harm. This includes physical aggression, sexual coercion, emotional abuse, and controlling behaviors[2].

Sexual Violence: this category includes any non-consensual sexual act or attempt to obtain a sexual act, as well as other actions targeting an individual's sexuality through coercion. Sexual violence can occur regardless of the relationship between the perpetrator and victim, and in any setting. A particularly severe form of sexual violence is rape, which is defined as the non-consensual penetration of the vulva or anus using the penis, other body parts, or objects, facilitated through physical force or coercion[3].

The World Health Organization (WHO) classifies violence against women as a significant public health issue due to its profound impact on women's physical and mental health. It compromises fundamental human rights, including the right to life, safety, dignity, and bodily integrity. The WHO equates the health consequences of violence to those of major diseases such as cancer, describing it as a leading cause of disability and death, comparable to the combined effects of malaria and traffic accidents[4].

IPV is further characterized by sustained patterns of ill-treatment within a relationship. The term "ill-treatment" refers to a habitual series of actions that harm the psycho-physical integrity, honor, or dignity of a family member or cohabitant. These actions may occur over an

[2] Centers for Disease Control and Prevention (CDC), *About Intimate Partner Violence | Intimate Partner Violence Prevention*, 2024, https://www.cdc.gov/intimate-partner-violence/about/index.html (20/10/2024).

[3] United Nations (UN), *Declaration on the Elimination of Violence against Women*, 1993, https://www.un.org/en/genocideprevention/documents/atrocity-crimes/Doc.21_declaration%20elimination%20vaw.pdf (20/10/2024).

[4] A. KUSTERMANN, A. UGLIETTI, F. CERIANI. *La violenza sessuale* in G. BOLIS (ed.) *Manuale di Ginecologia e Ostetricia*, Milano, 2011, pp. 709-717.

extended period but reflect a consistent pattern of abuse or denigration[5]. In contrast, isolated or sporadic incidents do not meet the threshold for the offense of mistreatment.

1.2. Prevalence and patterns of violence against women.

Sexual violence and IPV are globally pervasive phenomena, independent of cultural, religious, or socio-economic factors of both perpetrators and victims. Despite growing awareness of violence against women, scientific studies remain limited, and available data are often not comparable[6]. IPV and sexual violence are frequently combined in statistics, contributing to significant variability in reported prevalence.

Survivor-reported surveys provide the most accurate estimates of intimate partner and sexual violence. A 2018 WHO analysis of data from 161 countries found that nearly 1 in 3 women globally (30%) have experienced physical and/or sexual violence by an intimate or non-partner.

Over 25% of women aged 15–49 who have been in a relationship reported intimate partner violence at least once in their lifetime. Regional prevalence varies, with rates ranging from 20% in the Western Pacific to 33% in Africa and South-East Asia.

Globally, 38% of female homicides are committed by intimate partners, and 6% of women report sexual assault by non-partners,

[5] As stated in the Court of Cassation, criminal section VI, 20 January 2014 no. 2326.
[6] A. KUSTERMANN, cited above, p. 11.

though data on non-partner violence are limited. Violence is primarily perpetrated by men against women[7].

To better understand the scale of this issue, population-based surveys relying on victims' self-reports have been conducted, particularly in non-conflict areas. A 2006 Council of Europe survey revealed that one in five women across Europe had experienced sexual assault in their lifetime, with 98% of the perpetrators being male, and 50% being the victim's husband or partner[8].

The 2005 WHO Multi-country Study on Women's Health and Domestic Violence, conducted in 10 low- and middle-income countries, reported wide variation in the prevalence of intimate partner violence. In Japan, 15% of women reported experiencing physical and/or sexual violence by a partner, compared to 71% in Ethiopia. Additionally, between 0.3% and 11.5% of women reported experiencing non-partner sexual violence, with forced sexual initiation occurring for 17% of women in rural Tanzania, 24% in rural Peru, and 30% in rural Bangladesh[9].

A more recent analysis by the WHO, in collaboration with the London School of Hygiene and Tropical Medicine and the Medical Research Council, found that approximately 35% of women globally have experienced physical and/or sexual violence, whether by partners

[7] World Health Organization (WHO), *Violence Against Women Prevalence Estimates*, 2018, https://iris.who.int/bitstream/handle/10665/341338/9789240026681-eng.pdf (20/10/2024).

[8] Council of Europe, *Combating violence against women - Stocktaking study on the measures and actions taken in Council of Europe member States*, 2006, https://rm.coe.int/CoERMPublicCommonSearchServices/DisplayDCTMContent?documentId=090000168059aa52 (20/10/2024).

[9] World Health Organization (WHO), *WHO multi-country study on women's health and domestic violence against women*, 2005, https://www.who.int/publications/i/item/9241593512 (20/10/2024).

or non-partners[10]. Another analysis of IPV across diverse regions found similar trends: 22% of women in North London, 23% in Guadalajara, Mexico, 21.7% in Léon, Nicaragua, and 28.9% in Bangkok, Thailand, reported experiencing violence, with lower rates observed in places like Yokohama, Japan (6.2%). In high-income countries, the prevalence of lifetime partner abuse ranged from 8% in Canada to 14% in the UK and 7.7% in the United States[11].

Abrahams et al. conducted a comprehensive review of the global prevalence of sexual violence perpetrated by strangers. Despite the challenges in comparing data across studies, it was estimated that 7.2% of women over the age of 15 worldwide had reported experiencing sexual violence from strangers. The highest prevalence was observed in the central region of sub-Saharan Africa (21%). Additionally, the study found that in most countries, with the exception of Ethiopia and Japan, acquaintances were the most frequent perpetrators of sexual violence[12].

In the United States, estimates of IPV prevalence are drawn from the National Intimate Partner and Sexual Violence Survey (NISVS), an ongoing telephone survey initiated in 2010. The survey reveals that approximately 37.3% of women have experienced sexual violence, physical violence, or stalking by an intimate partner during their lifetime, and 23.2% of women have reported experiencing severe physical violence from an intimate partner[13].

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[10] World Health Organization (WHO), *WHO Prevalence and health effects of intimate partner violence and non-partner sexual violence*, 2013, https://www.who.int/publications/i/item/9789241564625 (20/10/2024).

[11] A. KUSTERMANN, cited above, p. 11.

[12] N. ABRAHAMS, *et al. Worldwide prevalence of non-partner sexual violence: a systematic review*, *Lancet*, 2014, 383, 9929, pp. 1648-1654.

[13] Centers for Disease Control and Prevention (CDC), National Intimate Partner and Sexual Violence Survey (NISVS): 2010-2012 state report, 2017, https://stacks.cdc.gov/view/cdc/46305 (20/10/2024).

While IPV occurs across all social, geographical, and cultural contexts, prevalence rates vary by demographic characteristics. Young adults (ages 18-24) exhibit higher rates of IPV violence compared to older age groups. Additionally, sexual and gender minorities, certain ethnic and racial minority groups, and individuals with mental or physical disabilities experience higher rates of IPV. This suggests a correlation between IPV and various forms of social marginalization. Furthermore, a link has been identified between intimate partner violence and human trafficking, where traffickers may initially pose as romantic partners before employing coercive and controlling tactics akin to those used in IPV[14].

In England, the 2013-2014 Crime Survey found that 2.2% of women aged 16-59 (approximately 355,000 women) had experienced some form of sexual violence, while 8.5% (1.5 million women) had experienced IPV in the preceding year (Office for National Statistics, 2013). Similarly, the 2015 Youth Risk Behavior Survey conducted among U.S. high school students revealed that 21.4% of female students had experienced physical or sexual violence from a partner in the previous year[15].

One notable finding from research into the epidemiology of intimate partner and sexual violence is the apparent stagnation in global data collection efforts. The most comprehensive global surveys on the prevalence of violence against women date back to 2018, with few updated datasets available on a worldwide scale. This lack of recent

[14] E. MILLER, B. MCCAW. *Intimate Partner Violence*, *N Engl J Med*, 2019, 380, 9, pp. 850-857.
[15] C.N. RASBERRY, *et al. Health-Related Behaviors and Academic Achievement Among High School Students - United States, 2015*. *MMWR Morb Mortal Wkly Rep*, 2017, 66, 35, pp. 921-927.

data presents a significant gap in understanding the current scope and trends of violence against women, particularly given the potential impacts of evolving socio-economic conditions, including the COVID-19 pandemic.

Within the European Union, efforts to update these estimates are underway through a survey initiated in 2020. However, only partial data from 2022 have been released[16], and the preliminary findings align closely with previous estimates[17]. While these efforts reflect a positive step toward more current data, the delay in comprehensive and updated information highlights the need for more regular and robust data collection processes. This is essential to assess the evolving dynamics of violence against women and to inform effective policy responses and intervention strategies.

Also, the impact of the COVID-19 pandemic on violence against women, particularly intimate partner violence, must be critically examined. The movement restrictions implemented to control the spread of the virus, such as lockdowns, exacerbated existing patterns of violence. During humanitarian crises and periods of displacement, pre-existing forms of violence, including intimate partner and non-partner sexual violence, often escalate, and new forms of violence may emerge.

Emergencies, including epidemics, tend to amplify violence against women, with particularly vulnerable groups including older women,

[16] Eurostat, EU survey on gender-based violence against women and other forms of inter-personal violence (EU-GBV) — first results 2022 edition Statistical reports, 2022, https://ec.europa.eu/eurostat/documents/7870049/15323622/KS-FT-22-005-EN-N.pdf/315d443b-ba8d-e607-3ce0-845f642a8c00?version=1.0&t=1669371271599 (20/10/2024).

[17] A. BARBIER, P. CHARIOT, T. LEFÈVRE. *Intimate partner violence against ever-partnered women in Europe: prevalence and associated factors-results from the violence against women EU-wide survey*. *Front Public Health,* 2022, 10, 1033465.

women with disabilities, refugees, and those displaced. Data from countries such as China, the United Kingdom, and the United States indicated a rise in IPV during the pandemic[18]. For instance, in Jingzhou[19], China, reports of intimate partner violence tripled in February 2020 compared to the same period in the previous year, highlighting the profound impact of the pandemic on violence within domestic settings.

Regarding Italian data, in 2006-2007, ISTAT conducted a survey via telephone interviews with a sample of 25,000 women aged 16 to 70. The results revealed that nearly one in three women (31.9%) had experienced at least one instance of physical and/or sexual violence in their lifetime. Specifically, 23.7% reported experiencing sexual violence, while 4.8% had been subjected to rape or attempted rape. In 69.7% of rape cases, the perpetrator was a partner, while 17.4% involved an acquaintance, and only 6.2% were committed by a stranger. Other types of assailants were reported less frequently.

Among women who are or have been in a relationship, 14.3% reported experiencing at least one act of physical and/or sexual violence by their partner. Of these, 27.2% sustained injuries as a result of the violence, and in 24.1% of cases, the injuries were severe enough to require medical treatment. The risk of violence is significantly higher

[18] M. GODIN, *As Cities Around the World Go on Lockdown, Victims of Domestic Violence Look for a Way Out*, Time, 2020, https://time.com/5803887/coronavirus-domestic-violence-victims/ (20/10/2024) and Women's Aid UK, *The Impact of COVID-19 on Women and Children Experiencing Domestic Abuse, and the Life-Saving Services that Support Them*, 2020, https://www.womensaid.org.uk/the-impact-of-covid-19-on-women-and-children-experiencing-domestic-abuse-and-thelife-saving-services-that-support-them/ (20/10/2024).

[19] B. ALLEN-EBRAHIMIAN, *China's Domestic Violence Epidemic*, Axios, 2020, https://www.axios.com/china-domestic-violencecoronavirus-quarantine-7b00c3ba-35bc-4d16-afdd-b76ecfb28882.html (20/10/2024).

for women whose partners exhibit certain behaviors. For example, 35.6% of women with physically violent partners experienced violence, compared to 6.5% of those without such partners. Similarly, if a partner is verbally abusive, the rate of violence rises to 25.7%, compared to 5.3% in women without an abusive partner. This pattern also extends beyond the family setting. Partners who devalue their spouses or exhibit low self-esteem further increase the likelihood of violence, with rates reaching 35.9%, compared to 5.7% among women without such partners.

Alcohol abuse also plays a significant role, as women with partners who drink to the point of drunkenness face an 18.7% risk of violence, compared to 6.4% for those whose partners do not drink excessively. Additionally, women whose fathers abused their mothers are at an elevated risk, with 30% reporting intimate partner violence compared to 6% of those without such a family history.

A significant barrier to accurately measuring the prevalence of violence is underreporting. For instance, 33.9% of women who experienced partner violence and 24% of those subjected to non-partner violence did not disclose their experiences to anyone[20].

In the emergency and urgent care units of Piedmont, Tuscany, and Sardinia, data collected since 2011 shows that approximately 1,300 women per 10,000 residents sought emergency care due to violence.

[20] L.L. SABADINI, *La violenza e i maltrattamenti contro le donne dentro e fuori la famiglia*, ISTAT, 2007, https://www.istat.it/it/files/2011/07/testointegrale.pdf (20/10/2024).

Over 70% of these women were aged 15-49, according to the European Union Injury Database[21].

Further surveys conducted by ISTAT in 2014 revealed that nearly one in three women, approximately 31.5%, have experienced at least one instance of physical and/or sexual violence in their lifetime. Specifically, 20.2% reported experiencing physical violence, 21% sexual violence, and 5.4% rape or attempted rape. Among the cases of rape, 62.7% of perpetrators were identified as partners, while 3.6% were relatives and 9.4% were friends. Additionally, 13.6% of women who are or have been in a relationship reported experiencing at least one form of physical and/or sexual violence from their partner[22].

It is important to note that statistical surveys conducted within the judicial system tend to underestimate the prevalence of violence, as they rely solely on incidents that victims choose to report. In Western countries, it is estimated that the reporting rate for such cases ranges from 5% to 50%. According to data from Tjaden et al. from the United States Department of Justice, only 36% of rapes, 34% of attempted rapes, and 26% of sexual assaults are reported to the relevant authorities[23].

In Italy, an alarming 91.6% of rape victims do not report the crime. Various factors contribute to this underreporting, including: fear of

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[21] M. GIUSTINI, *et al. European Injury Data Base (EU-IDB): data analysis 2020*, Rapporti ISTISAN, 2023, https://sante.public.lu/dam-assets/fr/publications/e/european-injury-database-eu-idb/european-injury-data-base-eu-idb-data-analysis-2020.pdf (20/10/2024).

[22] ISTAT, Il numero delle vittime e le forme della violenza, 2014, https://www.istat.it/it/violenza-sulledonne/il-fenomeno/violenza-dentro-e-fuori-la-famiglia/numero-delle-vittime-e-forme-di-violenza (20/10/2024).

[23] P. TJADEN, N. THOENNES, Full Report of the Prevalence, Incidence, and Consequences of Violence Against Women: Findings from the National Violence Against Women Survey, National Institute of Justice and the Centers for Disease Control and Prevention, 2000, https://www.ojp.gov/pdffiles1/nij/183781.pdf (20/10/2024).

retaliation from the aggressor; feelings of guilt for not being able to defend themselves; difficulty in recognizing the sexual violence experienced as a crime—particularly when the perpetrator is a spouse or partner; fear of not being believed; embarrassment about describing the incident; and the shame associated with undergoing a judicial process[24].

In relation to the Milan context, the case records from the Sexual and Domestic Violence Relief (SVSeD) of the Mangiagalli Clinic, an anti-rape center, at the Ospedale Maggiore Policlinico in Milan reveal the following data:

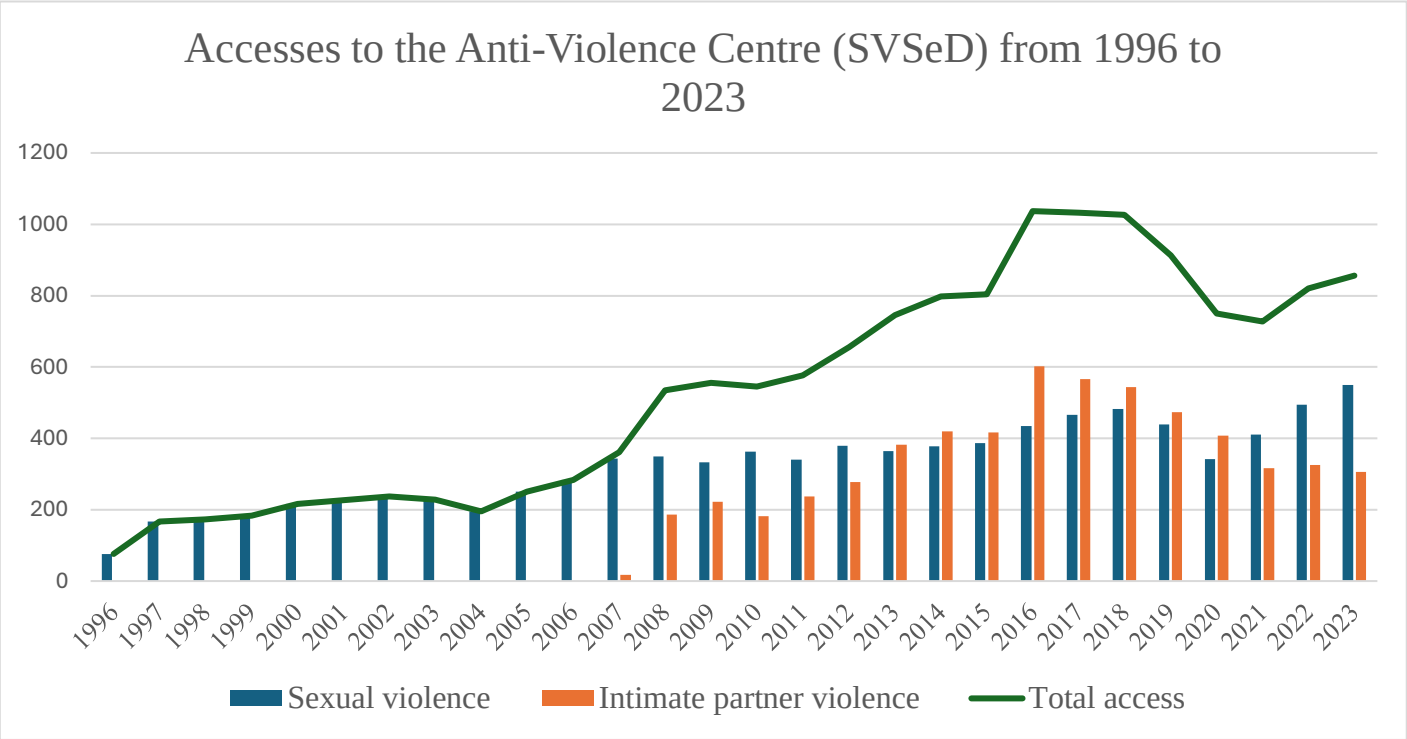
- In 2017, there were 1,032 visits: 466 for sexual violence and 566 for IPV.
- In 2018, there were 1,026 visits: 482 for sexual violence and 544 for IPV.
- In 2019, there were 914 visits: 420 for sexual violence and 494 for IPV.
- In 2020, there were 750 visits: 342 for sexual violence and 408 for IPV.
- In 2021, there were 728 visits: 411 for sexual violence and 317 for IPV.
- In 2022, there were 820 visits: 494 for sexual violence and 326 for IPV.
- In 2023, there were 856 visits: 550 for sexual violence and 306 for IPV.

[24] A. KUSTERMANN, cited above, p. 11.

For completeness, the trends in visits from 1996 to 2023 are depicted in the attached graph. A marked decrease in visits is evident after 2020, likely influenced by the Covid-19 pandemic, which resulted in a temporary rise in cases but has not yet returned to pre-pandemic levels. Notably, during the pandemic, many consultations occurred via telephone, limiting the possibility for in-person clinical intervention except in the most severe cases.

A significant development between 2021 and 2023 is the increase in sexual violence cases compared to IPV. This pattern diverges from the historically higher prevalence of IPV in Italy, suggesting a need for further investigation into the underlying causes. Global data indicate a rise in IPV during the pandemic, and the decrease in reported cases at the anti-violence center may reflect victims' reduced capacity to seek help or report incidents during this period[25].

[25] R. MAJEED-ARISS, *et al. The impact of COVID19 pandemic and government enforced lockdowns on levels of attendance and context of sexual assaults reported at Saint Marys Sexual Assault Referral Centre*, *J Forensic Leg Med*, 2023, 97, 102550; G. BARBARA, *et al. Intimate Partner Violence in the COVID-19 Era: A Health, Psychological, Forensic and Legal Perspective*, *Int J Environ Res Public Health*, 2022, 19, 9, 4973; A. VIERO, *et al. Violence against women in the Covid-19 pandemic: A review of the literature and a call for shared strategies to tackle health and social emergencies*, *Forensic Sci Int*, 2021, 319, 110650.



Accesses to the Anti-Violence Centre (SVSeD) from 1996 to 2023 (on the x-axis the years, on the y-axis the number of accesses)

1.3. Italian legal framework addressing violence against women.

The criminalization of violence against women is codified in the Italian Criminal Code under the section addressing crimes against personal freedom. This encompasses several distinct offenses. Notably, prior to 1996, offenses related to violence against women were classified as crimes against public morality and decency. However, following legislative reforms in 1996 (Law 15 February 1996 No. 66), these offenses were reclassified as crimes against the individual.

The two primary articles concerning sexual violence are Article 609 bis and 609 quarter of the Criminal Code, which address sexual violence and sexual acts involving minors. Sexual violence is defined by the violation of an individual's consent to a sexual act, thereby infringing upon the individual's right to personal autonomy and

freedom. Such abuse may occur through physical coercion, threats, or abuse of authority, whether public or private. Additionally, consent may be unlawfully obtained through deception or by exploiting the victim's mental, physical, or psychological vulnerability.

The term "*abuse of physical or psychological inferiority*" refers to instances where the perpetrator takes advantage of the victim's diminished capacity to exercise judgment and resistance, thereby manipulating their will. Importantly, this state of inferiority does not necessarily indicate the presence of a mental illness but may arise from other circumstances, such as a relationship of dependency, psychophysical immaturity, or the use of substances that impair the victim's ability to resist[26]. When the victim has a disability, this constitutes an aggravating circumstance. Deception through identity substitution is also recognized as a form of abuse[27].

Coercion may manifest in both physical and psychological forms and may involve forcing the victim to either perform or endure sexual acts. The degree of violence or threat need only be sufficient to overcome the victim's resistance; its severity is not a determining factor. Moreover, it is sufficient that the coercion occurs at the initiation of the act, as the victim may, due to physiological factors, become unable to continue resisting during the course of the crime.

[26] The Court of Cassation, Criminal Section IV, 31 October 2008, Judgment no. 40795, asserts that an offense is constituted not only by acts of coercion but also by any form of domination or suppression of the victim's will, even absent explicit intimidation or coercion, particularly where the victim's vulnerability is a factor.

[27] Deceptive impersonation extends beyond assuming the identity of individuals like a spouse; it also includes falsely attributing oneself with specific professional qualifications. See, in this regard, Court of Cassation, criminal section III, 21 September 2017 no. 43164.

To constitute a criminal offense, the violence must be the direct cause of the act, and the victim must be genuinely coerced and non-consenting. If the victim consents, even implicitly, to the violence—for instance, as a means of preserving appearances or to assuage personal scruples—the act does not meet the legal definition of sexual violence. To establish criminal intent in cases of sexual violence, it is sufficient to demonstrate the perpetrator's awareness and will to exert violence, threats, or abuse, along with knowledge of the sexual nature of the act and the victim's physical or psychological inferiority or the inducement of consent through deception. However, intent is excluded if the perpetrator is mistaken about the victim's physical or mental condition[28].

With respect to sexual acts involving minors, the law holds that such acts are criminal regardless of the minor's apparent consent. The invalidity of consent is directly linked to the minor's age. For minors under the age of 14, consent is considered absolutely invalid. For those between 14 and 16 years of age, consent is invalidated in cases where there is a relationship of authority, such as kinship or a "qualified non-parental relationship" which includes roles like educators, healthcare providers, or cohabitants. For example, an employer exploiting a maid in this context would fall under this provision[29]. It is crucial to note that a perpetrator cannot claim ignorance of the minor's age as a defense, as stated in Article 609 sexies of the Criminal Code.

[28] As explained in F. ANTOLISEI, *Manuale di diritto penale. Parte speciale, 15. ed., Milano, Giuffrè*, 2008, pp. 180-199.

[29] It is essential to underscore that in cases involving the induction of a child, particularly regarding the manipulation of a vulnerable and passively compliant child, the act constitutes a crime of sexual violence, as stated in Court of Cassation, criminal section III, 17 May 2018, no. 44530.

Nevertheless, consensual sexual relationships between minors are permissible provided the younger partner is at least 13 years old, and the age gap between the two is no greater than four years[30]. This provision represents a legal compromise designed to avoid the criminalization of relationships between young adolescents while preventing harmful sexual behavior. An aggravating circumstance arises when a sexual act is committed against a child under the age of ten.

The "corruption of a minor" is defined as exposing a child under 14 years of age to sexual acts with the intent of corrupting them (Article 609 quinquies of the Criminal Code)[31]. Importantly, the minor in such instances is considered a passive observer, not an active participant. If the minor is actively involved, the crime is classified as sexual assault or sexual acts with a minor. Furthermore, the child must be conscious and capable of perceiving the sexual acts for the offense to be considered valid; if the child is asleep, the offense does not exist, even if there is concern that the child might awaken. The mere fact that a conscious child witnesses a sexual act is insufficient to establish the crime unless there is a clear intent to expose the child to such acts for corruptive purposes[32].

Group sexual violence occurs when a sexual assault is carried out by at least two perpetrators. The legislature created a separate legal provision for this crime, with harsher penalties than those under Article

[30] Under Law 15 February 1996 No. 66, the period was initially set at three years, but it was later extended to four years by Law 19 July 2019 No. 69.

[31] There must be a specific intent to exploit a child (Court of Cassation, criminal section III, 15 April 2008 no. 15633), which can also be executed through video chat platforms (Court of Cassation, criminal section III, 11 May 2020 no. 14210).

[32] As explained in F. ANTOLISEI, cited above, p. 24.

609-bis of the Criminal Code, to address the growing concern over collective sexual offenses, particularly among younger individuals, which can manifest in ritualistic or orgiastic episodes. This offense places less emphasis on the psychological participation of individuals (such as intent, instigation, or reinforcement of criminal intent) and instead focuses on the dynamics of the group. A single act of participation is sufficient to constitute the offense if it is committed in the presence and function of the group. The requisite intent involves the perpetrator's conscious and voluntary participation in the sexual violence, alongside the other members of the group[33].

Aggravating circumstances, as outlined in Article 609-ter of the Criminal Code, include cases where at least one group member is a public official or holds a position of public authority. Mitigating circumstances may apply in cases where the individual's involvement in the crime was minimal or where the person was subjected to the direction or coercion of others to commit the offense.

The introduction of these provisions in the penal code redefined the legal framework concerning sexual acts, replacing the outdated concepts of "acts of lechery" and "carnal conjunction"[34]. This shift

[33] Group sexual violence encompasses both coercion and inducement, as established by the Court of Cassation, criminal section III, 11 October 1999 no. 11541. Furthermore, the involvement of multiple perpetrators is a fundamental element of this offense. It is important to note that mere participation, even without direct engagement in the sexual acts, suffices to establish culpability, as affirmed in Court of Cassation, criminal section III, 1 June 2000 no. 6464. Moreover, the members of the group may not necessarily witness the acts, as clarified in Court of Cassation, criminal section III, 25 March 2010 no. 11560. For more on these issues read M.N. MASULLO, *New Perspectives (and New Problems) in the Protection of Sexual Freedom: Group Violence, Cassazione Penale* 1997, pp. 2917 ff.

[34] Prior to its amendment, the Criminal Code categorized sexual offenses under the section entitled 'Offenses Against Public Morality,' specifically Articles 519 to 526 of the Criminal Code. This classification encompassed a range of offenses, including: rape and carnal intercourse committed under abuse of official authority; acts of violent lechery; rape with intent to compel marriage; rape for the purpose of lechery; rape of individuals below the age of fourteen or those deemed incapacitated, whether for lewd intent or forced matrimony; and seduction under false promises of marriage perpetrated by individuals already legally married.

eliminated the legal distinction between the presence or absence of penetration and instead focused on the violation of personal freedom concerning one's sexual autonomy. The concept of a sexual act involves physical contact between two individuals in bodily areas culturally recognized as having direct sexual resonance, calibrated to societal norms on a national level[35]. These areas include the genital region, the anal area, the mouth, and the breasts.

A sexual act is further understood as "any manifestation of the sexual instinct expressed in its various forms in which lust may be aroused"[36]. This definition encompasses both typical or physiological forms of copulation, such as vaginal intercourse, as well as non-normative sexual acts, including anal and oral intercourse, homosexual acts, and any other expressions of sexual desire[37]. It is crucial to note that the legal framework applies equally to individuals of any gender, including those engaged in prostitution, who retain the right to autonomous control over their bodies for sexual purposes[38].

It is noteworthy that acts of sexual violence may be perpetrated without physical presence, instead utilizing remote digital tools[39].

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[35] A. CADOPPI, *Commentary on Article 609 bis of the Criminal Code, in A. CADOPPI (ed.), Commentary on Laws Against Sexual Violence and Pedophilia, 4th ed., Cedam,* Padova, 2006, pp. 439 ff.

[36] The Constitutional Court 17 July 2000 no. 295 also addressed this issue, defining 'sexual acts' as the minimal threshold of behaviors indicative of violent lechery, thereby encompassing all forms of sexual aggression within this classification.

[37] For more on this topic and its links to the previous regulatory framework, see G. FIANDACA. *'Sexual Violence,' Encyclopedia of Law, supplement IV, Giuffré*, Milan, 2000, pp. 1153 ff; regarding the nature of sexual acts, Court of Cassation, criminal section III, 5 December 2005 no. 44246, determined that it is not requisite for the perpetrator to attain erotic satisfaction. Furthermore, as established in Court of Cassation, criminal section III, 17 Febbruary 2021 no. 6158, an overall assessment of the circumstances is imperative, particularly when the acts in question do not engage erogenous zones and consist of typical gestures of affection (e.g., a kiss on the cheek).

[38] As explained in F. ANTOLISEI, cited above, p. 24.

[39] See in this respect the Court of Cassation, criminal section III, 26 September 2012 no. 37076. concerning the conduct of a person who contacted underage girls via chat rooms in order to have pornographic photographs forwarded to him with repeated threats.

The issue of sexual violence also intersects with legislation on child prostitution, specifically Article 600-bis of the Criminal Code. This offense applies to minors between the ages of 14 and 18 and criminalizes both those who facilitate or profit from the prostitution of minors and those who engage in sexual acts with a minor in exchange for monetary or other material benefits. A single incident of such behavior is sufficient to constitute the offense.

For cases involving minors under the age of 14, the provisions of Article 609-quarter of the Criminal Code apply, while Article 609-bis of the Criminal Code governs cases where sexual intercourse occurs under conditions of violence, threat, or abuse.

Additionally, when addressing various forms of violence, it is important to consider the offense of ill-treatment, as outlined in Article 572 of the Criminal Code[40]. This article extends its protection not only to individuals under the authority of another person for purposes such as education, instruction, care, or custody, but also to "family members". Intimate partner violence is encompassed within the definition of ill-treatment and is consequently classified as a criminal offense. The previous legal notion that violence within a marital or intimate relationship might be justified for the purpose of fulfilling so-called "marital obligations" has been entirely abandoned.

The legal definition of family is broad, encompassing any group of individuals who, by virtue of emotional bonds or shared living

[40] The Article 572 of the Criminal Code was revised by Law 1 October 2012 No. 172, which ratified the Lanzarote Convention (25 October 2007) aimed at safeguarding children from sexual exploitation and abuse. Notably, this amendment expanded the definition of victims to include cohabitants, as well as imposing increased penalties for offenders. For further details, see: Court of Cassation, criminal section VI, 3 July 2013 no. 28603.

arrangements, have developed a relationship of mutual assistance and solidarity over a significant period. Notably, a legal relationship is not required between the victim and perpetrator for this offense to apply, as in the case of mistreatment involving a cohabitant's children.

The crime of ill-treatment also covers acts committed by individuals without a formal position of authority, such as a son mistreating his father or a grandson mistreating his grandfather. Ill-treatment includes any act that endangers legally protected interests such as personal safety, freedom, or honor. Moreover, this category of offenses is not limited to physical harm but also encompasses any action that causes moral or emotional suffering, including fear, anguish, or moral disgust, as well as acts of omission that generate such suffering.

Crucially, ill-treatment is considered a habitual offense, requiring a pattern of behavior rather than isolated incidents. The offense is completed when a single act of ill-treatment, whether active or passive, is followed by additional similar acts, reflecting the continuous nature of the abusive behavior. No harmful result or danger of illness is required. Consummation begins with the first vexatious act and continues until the end of the ill-treatment. The intent of the offence consists in the conscious intention to mistreat the victim, regardless of the end. The offences of insult, threat, beating and minor bodily harm are absorbed by the offence in question: only grievous bodily harm, grievous bodily harm and death are considered aggravating

circumstances, provided they are intentional[41]. Any other offences (e.g. sexual violence) are concurrent with ill-treatment[42].

Persecutory acts, commonly referred to as stalking, as defined in Article 612 bis of the Criminal Code[43], are considered habitual offenses. For the crime to be established, the threatening or violent behavior must be repeated at least once, and the incidents must be interconnected by a coherent and continuous context[44]. This conduct must result in at least one of the following: a persistent and severe state of anxiety or fear in the victim, a justifiable fear for their own safety or the safety of someone emotionally connected to them, or a compulsion to significantly alter their lifestyle.

The offences considered may be prosecutable ex officio (without a formal complaint from an injured party) or prosecutable on complaint by the offended person. Specifically, sexual violence, Article 609-bis of the Criminal Code, is prosecutable on complaint by the offended person, but becomes prosecutable ex officio if the victim is under 18

[41] For a more detailed explanation of the crime of ill-treatment see M. GAMBARDELLA, *Ill-treatment in the family or towards children,* in S. PREZIOSI (ed.), *Treatise on Special Part of Criminal Law. Crimes against the family, Italian Scientific Editions*, Rome, 2011, pp. 522 ff.

[42] The Italian legal framework has long grappled with the simultaneous application of the offences of sexual violence and ill-treatment, as jurisprudence has often sought to differentiate and address these crimes separately. However, prevailing interpretations now tend to separate sexual violence from the broader framework of ill-treatment, by proposing a division of charges when the act of violence is extraneous to and distinct from the habitual and repetitive nature of ill-treatment. In essence, acts of violence must align with the context of ill-treatment to be considered concurrently, when this overlap is absent, the two offences are treated independently, each resulting in distinct charges, see Court of Cassation, criminal section III, 14 December 2020 no. 35700. The repeated perpetration of acts of sexual violence is regarded as constituting ill-treatment, see Court of Cassation, criminal section III, 23 May 2005 no. 17843.

[43] This offence was not included in the 1996 reform and was introduced by Decree-Law no. 11 of 23 February 2009 (*Urgent measures on public security and combating sexual violence, as well as on stalking*), which was converted, with modifications, into Law no. 38 of 23 April 2009. It was further amended by Decree-Law no. 93 of 14 August 2013, converted into Law no. 119 of 15 October 2013.

[44] In this regard, see Court of Cassation, criminal section VI, 27 May 2003 no. 37019 affirming that acts of stalking must fall within a cohesive framework, where they are connected on an objective level by a pattern of habitual behavior, and on a subjective level by a unified criminal intent.

years of age or if the perpetrator is a parent, including an adoptive parent, cohabitee, foster parent or public official.

Sexual acts with a minor, Article 609-quarter of the Criminal Code, are prosecutable on complaint by the offended person, except in cases where the victim is under the age of 10 or if the perpetrator is a parent, including an adoptive parent, cohabiting partner, foster carer or public official.

Corruption of a minor, Article 609-quinquies of the Criminal Code, is always prosecutable ex officio, as is group sexual assault, Article 609-octies of the Criminal Code.

The offences indictable on complaint by the offended person mentioned above become indictable ex officio if they are committed in association with other indictable ex officio offences.

The offended person may file a complaint within twelve months of the act[45]: in fact, this is an exception that extends the right to sue by twelve months compared to the ordinary legislation. Another peculiarity is that the complaint is irrevocable, unlike the complaint under the ordinary legislation, to prevent the alleged offended person from withdrawing the complaint as a result of pressure and threats from the perpetrator of the offence, and on the other hand from using the complaint instrumentally.

Child prostitution, Article 600-bis of the Criminal Code, and ill-treatment against family members and cohabitants, Article 572 of the Criminal Code, are always prosecutable ex officio. Instead, prosecution of persecutory acts typically requires a formal complaint by the victim,

[45] Under Law 15 February 1996 No. 66, the period was initially set at six months, but it was later extended to twelve months by Law 19 July 2019 No. 69.

except in cases where the crime is committed against a minor or a person with disabilities.

Another milestone in the fight against violence is the Istanbul Convention, adopted on 11 May 2011 and ratified by Italy on 19 June 2013. The Convention's primary aim is to protect women from all forms of violence and to actively pursue its eradication. It presents a comprehensive framework not only to combat violence but also to ensure support, protection, and assistance for victims. Notably, Article 5 obligates states to refrain from acts of violence, particularly those committed by public officials, and mandates the implementation of legislative measures to investigate, prosecute perpetrators, and compensate victims. Additionally, Article 6 requires states to promote policies fostering integration, equality, and women's empowerment, while Article 36 encompasses definitions of violence and rape within its legal framework[46].

On 24 November 2017, a Decree issued by the President of the Council of Ministers established the National Guidelines for Health and Hospital Authorities regarding the rescue and socio-medical assistance of women victims of violence. These guidelines are applicable to all hospital facilities with emergency departments and aim to ensure a comprehensive and integrated approach to addressing the physical and psychological consequences of violence against women.

The intervention process begins with the woman's arrival at the emergency department, continuing through triage and referral to public

[46] Council of Europe Treaty Series - No. 210, Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence, Istanbul, 11 May 2011, https://www.istat.it/it/files/2017/11/ISTANBUL-Convenzione-Consiglio-Europa.pdf (20/10/2024).

or private services within the local territory. Key provisions include prioritizing emergency room access, ensuring medical examination within 20 minutes, and placing the victim in a protected environment. The guidelines also adopt recommendations from the Ministry of Health regarding medical examinations, as well as protocols for forensic DNA collection developed by the Scientific Association of Italian Forensic Geneticists (Ge.F.I.)[47].

Furthermore, the guidelines provide for the possibility of transferring the victim to an anti-violence center, with the activation of the local anti-violence network contingent upon the woman's consent. In cases where necessary for the woman's safety, hospital detention for up to 72 hours may be implemented.

In response to the increasing incidence of violence against women, including sexual violence, intimate partner violence, and feminicide, additional legislative measures have been enacted to address this pervasive issue. Law 19 July 2019 No. 69 commonly referred to as the "Code Red", was introduced to enhance the procedural protections available to victims of violent crimes, particularly those related to sexual violence and IPV. This law not only introduced new offenses into the Italian Penal Code but also imposed stricter penalties for crimes that affect women.

Among the newly established offenses is the crime of deforming an individual's appearance through permanent facial injuries (Article 583-quinquies of the Criminal Code), which explicitly criminalizes acts of

[47] GENETISTI FORENSI ITALIANI (Ge.F.I.), *Linee guida per la repertazione di tracce biologiche per le analisi di genetica forense nel percorso assistenziale delle vittime di violenza sessuale e/o maltrattamento*, 2018, https://www.gefi-isfg.org/temp/2202201374428.pdf (20/10/2024).

intentional disfigurement. Additionally, the law addresses the growing phenomenon of non-consensual dissemination of sexually explicit images or videos, commonly known as "revenge porn" (Article 612-ter of the Criminal Code). This provision extends liability not only to those who initially disseminate such material but also to those who subsequently share it, especially when the act is intended to harm the individuals depicted. The offense is further aggravated when committed within the context of a prior or ongoing emotional relationship, or with the use of digital tools[48].

Furthermore, Law 19 July 2019 No. 69 introduced the crime of coercion or inducement to marry (Article 558-bis of the Criminal Code), which carries aggravated penalties when the victim is a minor or when the crime is committed against an Italian citizen or foreign resident, even when perpetrated abroad[49]. Another significant addition is the criminalization of violations of orders requiring offenders to vacate the family home or prohibiting them from approaching locations frequented by the victim (Article 387-bis of the Criminal Code).

The law also intensifies the penalties for ill-treatment against family members or cohabitants (Article 572 of the Criminal Code), particularly

[48] This regulation was incorporated into the Law 19 July 2019 No. 69 in recognition of the dynamics between predominantly male perpetrators and predominantly female victims, who often experience such forms of violence following a decision to terminate their existing relationships, subsequently facing retribution from their ex-partners. Furthermore, this behavior can be attributed to the growing digitalization of contemporary society. For further details, see F. DI TANO, *Cyber Crimes and the Phenomena of Cyberstalking, Cyberbullying, and Revenge Porn,* in TH. CASADEI, S. PIETROPAOLI (eds.), *Law and Information Technology: Legal Informatics Issues, Institutional Perspectives, and Social Challenges*, Wolters Kluwer, Cedam Giuridica, Milan, 2021, pp. 165-178.

[49] The innovation of this measure lies in its international character, as it establishes an exception to the principle of territoriality in alignment with the stipulations of Article 44 of the 2011 Istanbul Convention. Thus, if this crime is perpetrated abroad by an Italian citizen or by a foreign national residing in Italy, the provisions of Article 558 bis of the Criminal Code will also be applicable. For further information see F. PICCIONI, *Abuse and Domestic Violence: The New Code Red and Defense Opportunities, in Legal series Maggioli* (ed.), Milan, 2024, pp. 27 ff.

when the offense occurs in the presence of a minor, a pregnant woman, or a person with disabilities, or when weapons are used. In such cases, the minor is always considered an offended party. Moreover, precautionary measures, such as restraining orders preventing the offender from approaching locations frequented by the victim, may be imposed on suspects of ill-treatment.

In addition to these new offenses, the law increases penalties for several crimes, including ill-treatment (Article 572 of the Criminal Code), sexual violence (Article 609-bis and subsequent articles of the Criminal Code), and sexual acts involving minors (Article 609-quater of the Criminal Code). An aggravating factor is introduced when such acts are carried out in exchange for money or other material benefits.

Moreover, Law 19 July 2019 No. 69 amends Article 165 of the Criminal Code, making the suspension of sentences for crimes of ill-treatment and violence against women contingent upon the offender's participation in social rehabilitation programs. This reform aims to promote the reintegration of offenders while simultaneously deterring recidivism.

Finally, the "Code Red" law introduces significant reforms in criminal procedure, prioritizing the swift prosecution of violent crimes to ensure quicker protections for victims. This procedural acceleration seeks to reduce delays in justice and enhance the effectiveness of the legal system in responding to cases of violence against women.

Specifically, the law mandates that upon receiving notice of an offense related to IPV or gender-based violence, the judicial police must promptly notify the public prosecutor, initially through an oral report,

to be followed immediately by a written one. The public prosecutor, within three days of the crime being registered, must gather information from the victim or the person who reported the crime, with this timeframe being extendable only under exceptional circumstances, such as protecting minors or preserving the confidentiality of the investigation, which may also serve the interests of the victim. Additionally, the judicial police must execute the investigative tasks delegated by the public prosecutor without delay and provide all relevant documentation promptly.

Further amendments to the Code of Criminal Procedure include provisions that specifically address the intersection of criminal and civil proceedings in cases of IPV and gender-based violence. The law stipulates that, when civil proceedings concerning spousal separation, child custody, or parental responsibility are underway, any criminal measures imposed in relation to IPV or gender-based violence must be promptly communicated to the civil court. Additionally, the law modifies the precautionary measure prohibiting the accused from approaching locations frequented by the victim, granting the court the authority to ensure compliance through electronic surveillance or other technical means, such as electronic bracelets. Furthermore, the law imposes an obligation on authorities to keep the victim and their legal counsel informed of significant developments, including the release of the accused, alterations to custodial or restrictive measures, instances of escape, or the issuance of orders prohibiting the accused from approaching the victim.

In addition to amending the Code of Criminal Procedure and the Criminal Code, Law 19 July 2019 No. 69 introduces provisions aimed at addressing the broader criminal justice framework. These include mandating specialized training programs for State Police, "Carabinieri", and Prison Police personnel involved in preventing and prosecuting IPV and gender-based violence crimes. The law also addresses the treatment of convicted offenders by amending the Prison Order (Law 26 July 1975 No. 354). It restricts the granting of prison benefits for individuals convicted of offenses involving permanent facial disfigurement until after a collective, scientifically-based personality assessment of at least one year. Furthermore, it allows convicted individuals of IPV and gender-based violence to undergo psychological treatment to support their rehabilitation, which may be considered when granting prison benefits. Lastly, the law designates the public prosecutor's office at the court, rather than at the Court of Appeal, as the competent authority for handling compensation claims when the crime occurred within the territory of an EU Member State, and the applicant resides permanently in Italy[50].

In the years that followed, additional legislative measures were implemented in response to the persistent and escalating phenomenon of violence, which, despite the initial efforts of lawmakers, continued to remain largely unchecked.

Law 27 September 2021 No. 134 introduced significant extensions to the protections afforded to victims of IPV and gender-based violence.

[50] For a more comprehensive analysis of the law and the supranational influences that shaped its formulation, refer to P. DI NICOLA TRAMAGLINI, F. MENDITTO. *Code Red, combating gender-based violence: from supranational sources to application instruments,in Giuffrè Francis Lefebvre* (ed.), The New Criminal Laws, Giuffrè, Milan, 2020.

It expanded the scope of the protections established by Law 19 July 2019 No. 69, extending them to include victims of attempted IPV and gender-based violence, as well as victims of attempted murder. Additionally, the law introduced a new provision mandating arrest in flagrante delicto for violations of restraining orders, including orders of removal from the family home and prohibitions on approaching places frequented by the victim.

Law 5 May 2022 No. 53 further strengthened the legal framework by enhancing the collection and analysis of statistical data related to gender-based violence. This law introduced several key measures: it obligates offices, agencies, organizations, and both public and private entities involved in official statistical reporting to collect, process, and provide disaggregated data by gender for national surveys. Moreover, public health institutions, particularly emergency departments, are required to provide data on incidents of violence against women. The law also established an integrated system between the Ministries of the Interior and Justice for collecting data on offenses related to violence against women, with a specific focus on information that reveals the relationship between the perpetrator and the victim.

Furthermore, the law mandates adjustments to the collection of data concerning specific offenses, ensuring the recording of information such as the relationship between the perpetrator and the victim, their age and gender, and the circumstances surrounding the offense. Finally, the law enhances annual surveys conducted by ISTAT, enriching the data concerning services and resources provided by anti-violence

centers and shelters, thereby improving the overall understanding and response to gender-based violence in Italy.

Law 24 November 2023 No. 168 introduces significant amendments to the Criminal Code and the Code of Criminal Procedure, along with new preventive measures aimed at enhancing the effectiveness of efforts to combat violence against women. Key provisions include the extension of the police commissioner's warning to cover offenses such as private violence, aggravated threats, stalking, unlawful dissemination of sexually explicit images or videos, trespassing, and damage to property. The law also expands the definition of ill-treatment to include "witnessed violence," referring to acts of violence committed in the presence of minors. Furthermore, the prefect is empowered to implement dynamic surveillance measures when there is a risk of offenses related to gender violence or IPV, particularly where there is a danger of repeated offenses. Additionally, in cases where the offender has already received a prior warning, all subsequent acts of violence are subject to ex officio prosecution, accompanied by increased penalties.

In terms of preventive measures, the law extends the application of the "Anti-Mafia" Code to include personal preventive measures for individuals convicted of serious crimes related to violence against women, allowing for special surveillance, including the use of electronic monitoring devices such as bracelets. Other preventive actions include restrictions on approaching specific locations and a mandatory distance of at least 500 meters from protected individuals or places. Violations of these protection orders result in imprisonment.

The law also addresses judicial procedures by prioritizing cases related to violence against women. This priority applies to both trial hearings and precautionary measures, with strict deadlines: public prosecutors must assess precautionary measures within 30 days, and judges must issue decisions on their validation within 20 days. Compliance with these timelines is monitored quarterly. Additionally, when substantial evidence exists against a suspect of offenses related to violence against women, the public prosecutor can order the immediate removal of the suspect from the family home and impose a prohibition on approaching places frequented by the victim.

Further innovations introduced by the law include imprisonment for tampering with electronic monitoring devices, and a requirement for authorities to promptly notify victims of any restrictive measures imposed on the offender or their revocation, with the possibility of implementing dynamic surveillance. Conditional suspension of the offender's sentence must be accompanied by successful completion of rehabilitation programs, and preventive measures will also be assessed by public security authorities. Additionally, the law facilitates victim compensation by allowing the judge to grant a provisional sum of money in advance, and it also provides for the recognition and accreditation of organizations and associations focused on the rehabilitation of perpetrators of violence.

In 2023, two other significant laws related to violence against women were also enacted. Law 17 February 2023 No. 12 established a bicameral commission of inquiry on femicide and all forms of gender-based violence. Law 8 September 2023 No. 122 mandates that the

public prosecutor obtain information from the victim or the individual reporting the crime within three days of the crime's registration. Failure to meet this deadline results in the magistrate's replacement.

1.4. Clinical presentations and health impacts of violence against women.

Violence has a profound and detrimental impact on physical, mental, sexual, and reproductive health, resulting in both immediate and long-term consequences for survivors and their children. The economic and social costs of such violence are substantial. Sexual violence, in particular, inflicts a deep psychological wound, the effects of which persist over time unless properly addressed through adequate support and intervention. Unlike other forms of trauma, sexual violence directly affects an individual's bodily autonomy and sexual sphere, undermining self-esteem, trust in others, emotional well-being, and intimate relationships[51].

Violence against women can result in fatal outcomes, including homicide and suicide, as well as serious injuries. For example, 42% of women who reported being raped sustained physical injuries as a result of the assault[52]. Additional health impacts include chronic pain conditions such as headaches, abdominal and back pain, fibromyalgia, gastrointestinal disorders, and decreased overall health.

Furthermore, violence often leads to unintended pregnancies, induced abortions, gynecological issues, and sexually transmitted

[51] A. KUSTERMANN, cited above, p. 11.
[52] World Health Organization (WHO), 2024, cited above, p. 10.

infections (STIs), including HIV. A 2013 analysis by the WHO[53] found that women who had experienced physical or sexual violence were 1.5 times more likely to contract STIs and, in some regions, HIV, compared to women who had not been raped. These women were also twice as likely to have an abortion. Ill-treatment during pregnancy increases the risk of miscarriage, pre-eclampsia, pre-term delivery, intrauterine death, and low birth weight. Additionally, substance abuse, smoking, and the infrequent use of prenatal care are often linked to pregnancy complications in cases of partner violence. Globally, partner violence is the second leading cause of death in pregnant women, surpassed only by hemorrhage[54].

The psychological toll of violence is equally severe. Survivors are at an increased risk of developing depression, PTSD,[55] insomnia, eating disorders, emotional disorders, and suicidal ideation. According to WHO women who experienced IPV were twice as likely to suffer from depression and alcohol dependency[56]. The risk of mental health issues is further exacerbated for those who have endured non-domestic sexual assault. Childhood sexual abuse has been identified as a significant risk factor for developing eating disorders such as anorexia and bulimia. Additionally, research has demonstrated a correlation between chronic pelvic pain without an identifiable organic cause and previous sexual

[53] World Health Organization (WHO), 2013, cited above, p. 14.

[54] L. HEISE, M. ELLSBERG, M. GOTTEMOELLER, *Ending Violence Against Women. Population Reports, Series L, No. 11. Baltimore*, John Hopkins University School of Public Health, Population Information Program, 1999, https://vawnet.org/material/population-reports-ending-violence-against-women.

[55] D.E. STEWART, S.N. VIGOD. *Update on mental health aspects of intimate partner violence*, *Med Clin North Am,* 2019, 103, pp. 735–749.

[56] World Health Organization (WHO), 2024, cited above, p. 10.

trauma[57]. Women with a history of abuse are also more likely to experience chronic gastrointestinal disorders[58], dysmenorrhea, and dyspareunia[59]. Additionally, victims of violence face an elevated risk of developing cardiovascular diseases, metabolic disorders, and certain types of cancer.[60]

Sexual violence, particularly when experienced during childhood, is associated with increased likelihood of substance abuse, including cigarettes, drugs, and alcohol, as well as engagement in risky sexual behaviors later in life. According to the "*World Report on Violence and Health*"[61], childhood sexual abuse accounts for 4-5% of cases of alcoholism, drug addiction, and depression among men, and 7-8% among women. Additionally, exposure to violence is correlated with an increased propensity for men to become perpetrators of violence and for women to become victims.

The 2007 ISTAT survey of Italian women revealed that women who experienced repeated episodes of violence from their partners often suffered significant psychological and emotional consequences. Nearly half reported a loss of self-confidence and reduced self-esteem, while 44.9% experienced feelings of helplessness, 41.5% had sleep

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[57] M. HILDEN, *et al. A history of sexual abuse and health: a Nordic multicentre study*, *BJOG*, 2004, 111, 10, pp. 1121-1127.

[58] N. PALLOTTA, *et al. Prevalenza dei maltrattamenti fisici e/o sessuali in pazienti con disturbi cronici gastrointestinali*, *Neurogastroenterologia*, 1998, 3, pp. 102-106.

[59] E.A. WALKER, *et al. Adult health status of women with histories of childhood abuse and neglect*, *Am J Med*, 1999, 107, 4, pp. 332-339.

[60] J.S. CHANDAN, *et al. Risk of cardiometabolic disease and all-cause mortality in female survivors of domestic abuse*, *JAMA*, 2020, 9, e014580; A. STUBBS, C. SZOEKE, *The effect of intimate partner violence on the physical health and health-related behaviors of women: a systematic review of the literature, Trauma Violence Abuse,* 2022, 23, pp. 1157–1172; J.M. REINGLE GONZALEZ, *et al. Violence against women increases cancer diagnoses: results from a meta-analytic review*, *Prev Med*, 2018, 114, pp.168–79.

[61] E.G. KRUG, *et al. The world report on violence and health*, *Lancet*, 2002, 360, 9339, pp. 1083-1088.

disturbances, 37.4% experienced anxiety, 35.1% suffered from depression, 24.3% reported difficulties with concentration and attention, 18.5% experienced chronic pain in at least three different areas of the body, 14.3% struggled with parenting, and 12.3% had suicidal or self-harming thoughts[62].

Children who witness ill-treatment are at high risk of developing emotional and behavioral disorders, with many exhibiting patterns of violent behavior in their own interpersonal relationships. For girls, exposure to violence often results in the development of dependent, passive behaviors and difficulties in self-protection, which may perpetuate a cycle of victimization throughout their lives. Moreover, IPV has been linked to increased pediatric mortality rates, often due to conditions such as gastrointestinal illnesses and malnutrition[63].

Victims of sexual violence may present to healthcare professionals with a wide range of symptoms, which vary depending on the context of the assault, the identity of the perpetrator, and the personal history of the victim. As noted by Kustermann et al.[64], violence may be perceived as a singular, life-threatening event, or as part of a pattern of repeated abuse, leading to emotional numbness over time. Symptoms may include acute or chronic PTSD, or other psychological manifestations such as anxiety, fear, depression, emotional instability, passivity, partial or complete amnesia, incoherent speech, confusion, drowsiness, emotional detachment, and aggression.

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[62] L.L. SABADINI, cited above, p. 18.

[63] R. JEWKES, M. MACHISA. *Maternal intimate partner violence and child health outcomes. Lancet Glob Health.* 2024, 12, 11, pp. 1740-1741.

[64] A. KUSTERMANN, cited above, p. 11.

Victims frequently report a diminished self-image, characterized by feelings of worthlessness and powerlessness. They may express guilt, shame, and inferiority for not having been able to defend themselves against the aggressor. Victims may also present as disoriented, confused, and unable to recall the details of the trauma. Additionally, they may suffer from heightened anxiety and distress related to the threats they received and exhibit profound fear for their personal safety and well-being. In some cases, they may experience self-destructive impulses, including suicidal ideation.

Chapter II
THE EPIGENETIC PERSPECTIVE ON VIOLENCE AGAINST WOMEN

Contents: 2.1 Introduction to epigenetics. 2.2 Epigenetic markers associated with childhood ill-treatment. 2.3 The relationship between sexual violence and post-traumatic stress disorder. 2.4 Epigenetic mechanisms underlying post-traumatic stress disorder.

2.1. Introduction to epigenetics.

The term "epigenetics" refers to both heritable changes in gene activation and expression (inherited by progeny cells or individuals) and stable, long-term modifications in a cell's transcriptional potential that are not necessarily passed down genetically[65]. In essence, these are modifications at the chromosomal level that can be inherited but are not the result of alterations in the DNA sequence itself[66].

The collection of chemical molecules that modify DNA and its associated structures forms the epigenome, which encompasses any modifier of gene expression independent of the DNA sequence. While the genome represents the complete set of genetic material encoded in DNA within an organism's cells, the epigenome includes the array of complex modifications associated with genomic DNA, contributing to the unique and specific identity of each cell type during development or disease[67].

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[65] S.L. BERGER, *et al. An operational definition of epigenetics*, *Genes Dev*, 2009, 23, 7, pp. 781-783.
[66] T. STRACHAN, A.P. READ. *L'espressione dei geni umani* in G.A. DANIELI (ed.) *Genetica molecolare umana*, Milano, 2006, pp. 278-283.
[67] C.M. RIVERA, B. REN. *Mapping human epigenomes*, *Cell*, 2013, 155, 1, pp. 39-55.

The epigenome plays a crucial role in integrating genomic information with molecular and chemical signals from cellular, extracellular, and environmental sources. It defines the unique gene expression patterns of each cell type, which in turn determines its functional identity. Furthermore, the epigenome reflects an organism's capacity to adapt and evolve by expressing phenotypic traits that have emerged in response to environmental stimuli.

Unlike the relatively static nature of the genome, the epigenome is highly dynamic and adaptable, responding to intracellular and extracellular stimuli. These stimuli may include interactions between neighboring cells, physiological changes, or environmental factors. Environmental influences on the epigenome are diverse and can either promote health and well-being or lead to adverse effects. Positive influences include physical exercise, beneficial gut microbiota (the microbiome), and certain forms of alternative medicine[68].

Conversely, harmful factors include exposure to toxic substances and the abuse of drugs. Other factors, such as diet, seasonal changes, socioeconomic status, psychological well-being, social interactions, medical treatments, and exposure to diseases, can have both positive and negative effects on the epigenome, potentially influencing health outcomes or contributing to the development of disease or psychological disorders.

Cytokines, growth factors, hormones, and neurotropic substances, along with other stress-response molecules, are among the various

[68] R.R. KANHERKAR, N. BHATIA-DEY, A.B. CSOKA. *Epigenetics across the human lifespan, Front Cell Dev Biol*, 2014, 2, pp. 49.

environmental factors that modulate the epigenome. Changes in the levels of these molecules, influenced by environmental conditions, contribute to the modification of the epigenome. An individual's unique epigenetic and genetic profile determines their specific response to these environmental factors.

Environmental stimuli can alter enzyme activity, leading to the addition or removal of epigenetic marks on DNA or chromatin, resulting in transient, permanent, or even heritable changes in cellular memory[69]. Although every cell in an organism contains an identical genome, the phenotype is not fixed. Phenotypic changes are driven by alterations in gene expression in response to environmental cues.

The primary mechanisms through which these epigenetic modifications occur include DNA methylation, histone modification, and RNA-associated gene silencing. The methylome, which refers to the genome-wide distribution of methylated DNA sequences within a cell, is subject to modification depending on environmental factors and the stage of cellular development[70].

DNA methylation involves the covalent addition of a methyl group to the fifth carbon of the pyrimidine ring of cytosine, creating 5-methylcytosine (5mC). In mammals, gene transcription often begins at promoters that are rich in CpG sites, which consist of cytosines positioned near phosphate-linked guanine nucleotides. These CpG-rich regions, known as CpG islands, play a critical role in regulating gene expression. In the human genome, 60-80% of the 28 million CpG

[69] Ibidem

[70] M.J. ZILLER, *et al. Charting a dynamic DNA methylation landscape of the human genome, Nature*, 2013, 500, 7463, pp. 477-481.

dinucleotides are methylated[71]. Chromatin structures surrounding CpG island-rich promoters are typically open and accessible, facilitating gene transcription. However, when CpG islands become methylated, the chromatin compacts tightly, repressing gene transcription and preventing gene expression.

In active CpG islands, cytosines are typically unmethylated. When spontaneous deamination of unmethylated cytosine to uridine occurs, DNA repair mechanisms convert the uridine back to cytosine, highlighting the evolutionary significance of preserving CpG sequences. The presence of 5-methylcytosine (5mC) within a CpG island indicates promoter inactivity, resulting from chromatin condensation driven by DNA methylation.

Methylation of CpG sites is carried out by a group of enzymes known as DNA methyltransferases (DNMTs). These enzymes play crucial roles in establishing and maintaining methylation patterns, especially during embryogenesis. DNMT1 is responsible for preserving methylation patterns across cell divisions, ensuring that they are faithfully copied during cell replication. DNMT2 is associated with embryonic stem cells and may play a role in RNA methylation. DNMT3a and DNMT3b are involved in de novo methylation, establishing new methylation patterns at CpG sites[72].

Recent studies have shown that cytosine methylation can also occur at non-CpG sites, such as CpA, CpC, and CpT, particularly in neuronal

[71] Ibidem

[72] S. SINGH, S.S. LI. *Epigenetic effects of environmental chemicals bisphenol A and phthalates, Int J Mol Sci*, 2012, 13, 8, pp. 10143-10153.

cells[73]. DNA demethylation is a complex process involving at least two enzymatic pathways: base excision repair (BER) and oxidation. BER is facilitated by protein complexes, including the GADD45 family of structural proteins, the AID/APOBEC family of cytosine deaminases, and the methyl-DNA binding protein MBD4. Oxidation is mediated by the TET family of proteins (TET1-3), which further modulate the demethylation process.

Histones, which serve as the core structural proteins of the chromatin complex, provide the scaffolding around which DNA is tightly coiled at regular intervals to form chromatin. The nucleosome, the fundamental unit of chromatin organization, consists of an octameric core made up of two copies of histones H2A, H2B, H3, and H4, around which DNA is wrapped[74]. Histones play a crucial role in regulating DNA packaging, influencing the degree of chromatin compactness, and thereby controlling both transcriptional activity and transcriptional silencing.

Histone modifications refer to posttranslational changes occurring on the flexible N- and C-terminal tails of histones that extend from the globular histone octamer. These modifications include acetylation of lysine residues, methylation of lysine and arginine residues, phosphorylation of serine and threonine residues, ubiquitination of lysine residues, as well as sumoylation and ADP-ribosylation. These chemical changes collectively influence DNA transcription. The addition or removal of methyl groups on DNA and histones, alongside

[73] R. LISTER, *et al. Human DNA methylomes at base resolution show widespread epigenomic differences,* Nature*,* 2009, 462, 7271, pp. 315-322.

[74] K. LUGER, *et al. Crystal structure of the nucleosome core particle at 2.8 Å resolution, Nature*, 1997, 389, 6648, pp. 251-260.

acetyl groups on histones, represent key mechanisms of epigenetic modification[75].

Histone acetylation is catalyzed by enzymes known as histone acetyltransferases (HATs), which add acetyl groups to lysine residues on histone tails. In contrast, histone deacetylases (HDACs) remove acetyl groups from acetylated lysines. Acetylation typically results in a "relaxed" chromatin state, enhancing transcriptional activity of the associated genes, whereas deacetylation leads to chromatin condensation and transcriptional repression.

RNA-associated silencing is a post-transcriptional gene regulation mechanism that suppresses or reduces gene expression through small non-coding RNA molecules, such as microRNAs (miRNAs) and small interfering RNAs (siRNAs). Despite miRNAs representing only 1% of the genome, it is estimated that they target about 30% of genes[76]. These small RNAs function as regulatory switches and modulators within cells, fine-tuning gene expression in a cell-type-specific manner during development and in pathological conditions[77]. miRNAs are involved in processes such as tumor suppression, apoptosis, cell proliferation, and motility[78]. RNA silencing mechanisms, such as miRNA-mediated silencing, often involve non-coding RNAs binding to the 3' untranslated

[75] H. CEDAR, Y. BERGMAN. *Linking DNA methylation and histone modification: patterns and paradigms, Nat Rev Genet*, 2009, 10, 5, pp. 295-304.

[76] B.P. LEWIS, C.B. BURGE, D.P. BARTEL. *Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell*, 2005, 120, 1, pp. 15-20.

[77] C. BAER, R. CLAUS, C. PLASS. *Genome-wide epigenetic regulation of miRNAs in cancer, Cancer Res*, 2013, 73, 2, pp. 473-477.

[78] R. KALA, *et al. MicroRNAs: an emerging science in cancer epigenetics, J Clin Bioinforma,* 2013, 3, 1, pp. 6.

regions (3' UTR) of target mRNAs, leading to mRNA degradation and downregulation of gene expression[79].

All genes, regardless of cell type, are regulated through the interaction of various epigenetic mechanisms, which either activate or silence gene expression. These mechanisms may result in widespread changes, such as global shifts in DNA methylation affecting numerous genes, or more localized changes, altering the expression of specific individual genes. However, the precise cellular pathways and mechanisms that govern these global or specific epigenetic alterations remain largely unknown.

A theoretical framework has been proposed to explain how epigenetic factors might influence gene expression. This framework suggests that epigenetic factors can exert their effects through direct or indirect mechanisms. The direct mechanism can be further divided into two types: Type 1 and Type 2.

The Type 1 direct effect involves the epigenetic factor directly altering the activity of epigenetic enzymes. This may occur through various pathways, such as the epigenetic factor binding to the enzymes, inhibiting their normal function, damaging them, or increasing their production. These disruptions in the bioavailability of epigenetic enzymes lead to the improper recruitment of epigenetic modifiers, such as those involved in DNA methylation and histone modification, to gene promoters and enhancers across the genome. For example, the

[79] S.K. SINGH, *et al. MicroRNAs—micro in size but macro in function, FEBS J*, 2008, 275, 20, pp. 4929-4944.

antihypertensive drug hydralazine inhibits DNA methylation by directly interacting with DNA methyltransferases.

In the Type 2 direct effect, an epigenetic factor influences biochemical pathways, altering the availability of substrates required for the synthesis of epigenetic molecules. For instance, an epigenetic factor may affect the levels of acetate, which is necessary for histone acetylation. Such changes result in the altered availability of epigenetic molecules, leading to nonspecific modifications across the epigenome. These modifications may have far-reaching consequences for gene regulation, as they impact chromatin structure and gene expression at a broad level.

The second primary mechanism by which external factors induce epigenetic modifications is referred to as the indirect mechanism. This process is thought to occur through a biphasic response. Initially, acute exposure to a factor affects various cell signaling pathways, leading to changes in the expression of growth factors, receptors, and ion channels. These alterations influence transcription factor activity on gene promoters, modulating gene expression. However, with prolonged or chronic exposure, transcription factors and other gene regulatory proteins not only change gene expression but also interact with epigenetic enzymes. These enzymes are either recruited to or rejected from specific chromatin regions, which results in the addition or removal of epigenetic markers. In this manner, cells adapt to sustained changes in gene expression, leading to long-term modifications in DNA methylation and chromatin structure, thereby producing a lasting reconfiguration of the epigenetic network. An example of this process

can be observed with isotretinoin, a drug that acts as a transcriptional factor, leading to such epigenetic alterations.

Throughout an individual's life, numerous epigenetic changes occur in response to environmental interactions, and these changes are integrated chronologically. In differentiated cells, signaling pathways regulate cellular functions, allowing for the flexible adaptation of the epigenome to environmental changes and personal experiences. This flexibility permits the epigenome to serve as the "software" of the genome[80], guiding embryonic and prenatal development as well as postnatal growth and maturation. Specific gene groups are epigenetically activated or silenced during different stages of life, governing individual development and maturation in response to environmental stimuli and genetic predispositions.

However, this flexibility, while beneficial in facilitating adaptation to environmental conditions, also introduces vulnerabilities. Epigenetic factors influencing genes or the broader gene expression network throughout an individual's life can disrupt regulatory processes, leading to long-term consequences. These imbalances may result in diseases and negative outcomes, both at the individual level and across human evolution.

2.2. Epigenetic markers associated with childhood ill-treatment.

Childhood ill-treatment and adverse early-life experiences are widely recognized as nonspecific risk factors for the development of various psychiatric disorders in adulthood, including PTSD, bipolar disorder,

[80] R.R. KANHERKAR, cited above, p. 47.

depression, anxiety disorders, eating disorders, psychotic disorders, substance use disorders, personality disorders, and elevated suicide risk.[81] Additionally, childhood ill-treatment has been implicated in increased susceptibility to non-psychiatric conditions such as ischemic heart disease, stroke, respiratory diseases, diabetes, and cancer[82].

Epigenetic modifications have been proposed as a potential mechanism linking early adverse experiences to long-term health outcomes[83]. Due to heightened brain plasticity during developmental stages, children are particularly vulnerable to the negative impacts of trauma, which may lead to epigenetic changes. These alterations can result in both structural and functional changes in brain development, contributing to long-lasting effects on cognitive and behavioral capacities[84].

DNA methylation is the most extensively studied epigenetic mechanism in the context of psychiatric disorders[85]. Research has focused on both gene-specific methylation and genome-wide methylation patterns. Several studies have explored methylation

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[81] L.P. CHEN, *et al. Sexual abuse and lifetime diagnosis of psychiatric disorders: systematic review and meta-analysis, Mayo Clin Proc*, 2010, 85, pp. 618-629; S. JONAS, *et al. Sexual abuse and psychiatric disorder in England: results from the 2007 Adult Psychiatric Morbidity Survey, Psychol Med*, 2011, 41, pp. 709-719; M.J. ALVAREZ, *et al. Prevalence and clinical impact of childhood trauma in patients with severe mental disorders, J Nerv Ment Dis*, 2011, 199, pp. 156-161; K.A. MCLAUGHLIN, *et al. Childhood adversities and adult psychopathology in the National Comorbidity Survey Replication (NCS-R) III: associations with functional impairment related to DSM-IV disorders, Psychol Med,* 2010, 40, pp. 847-859; C.P. CARR, *et al. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes, J Nerv Ment Dis*, 2013, 201, pp. 1007-1020.

[82] V.J. FELITTI, *et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study, Am J Prev Med*, 1998, 14, pp. 245-258.

[83] J.P. SHONKOFF, W.T. BOYCE, B.S. MCEWEN. *Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention, JAMA*, 2009, 301, pp. 2252-2259.

[84] T.L. ROTH, *et al. Lasting epigenetic influence of early-life adversity on the BDNF gene, Biol Psychiatry*, 2009, 65, pp. 760-769.

[85] J. NÖTHLING, *et al. Epigenetic alterations associated with childhood trauma and adult mental health outcomes: A systematic review, World J Biol Psychiatry*, 2019, pp. 1-20.

changes in healthy adult individuals with a history of childhood trauma. Notably, methylation alterations have been observed in genes encoding the glucocorticoid receptor (NR3C1)[86], interleukin-6 (IL-6)[87], the oxytocin receptor (OXTR), and brain-derived neurotrophic factor (BDNF)[88].

In one study, childhood ill-treatment was associated with increased methylation in the serotonin transporter gene (SLC6A4) promoter region in lymphoblast DNA, a gene linked to several neuropsychiatric conditions[89]. Another investigation identified methylation differences at four CpG sites in non-promoter regions of SLC6A4 in individuals with a history of childhood trauma[90]. However, studies on SLC6A4 have yielded inconsistent findings[91]. Furthermore, methylation profiles of the FKBP5 and SKA2 genes have not shown significant associations between childhood trauma and the development of PTSD[92].

McGowan et al. observed hypermethylation in the neural growth factor-induced protein A (NGFI-A) binding site within the glucocorticoid receptor variant 1F promoter in suicidal individuals with

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[86] B. LABONTÉ, *et al. Genome-wide epigenetic regulation by early-life trauma, Arch Gen Psychiatry*, 2012, 69, pp. 722-731.

[87] L.W. JANUSEK, *et al. Relationship of childhood adversity and neighborhood violence to a proinflammatory phenotype in emerging adult African American men: An epigenetic link, Brain Behav Immun*, 2017, 60, pp. 126-135.

[88] E. UNTERNAEHRER, *et al. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women, Stress,* 2015, 18, pp. 451-461.

[89] S.R.H. BEACH, *et al. Methylation at SLC6A4 is linked to family history of child abuse: an examination of the Iowa Adoptee sample, Am J Med Genet B Neuropsychiatr Genet*, 2010, 153B, pp. 710-713.

[90] M. VIJAYENDRAN, *et al. Effects of genotype and child abuse on DNA methylation and gene expression at the serotonin transporter, Front Psychiatry*, 2012, 3, pp. 55.

[91] M. WANKERL, *et al. Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles, Transl Psychiatry*, 2014, 4, pp. e402.

[92] M.P. BOKS, *et al. SKA2 Methylation is Involved in Cortisol Stress Reactivity and Predicts the Development of Post-Traumatic Stress Disorder (PTSD) After Military Deployment, Neuropsychopharmacology*, 2016, 41, pp. 1350-1356.

a history of childhood ill-treatment. This hypermethylation is associated with reduced expression of the glucocorticoid receptor in the hippocampus[93]. Moreover, increased methylation in ribosomal RNA gene promoters was detected in these individuals, which correlates with decreased gene expression in the hippocampus[94].

In ill-treated children, there are disruptions in neurogenesis and synapse architecture in the hippocampus, along with reduced neuroplasticity. One gene implicated in neuroplasticity is ALS2, which encodes two protein isoforms that regulate small GTPase activity involved in various cellular functions, including chromatin condensation, cytoskeletal organization, neuronal morphogenesis, and axon and dendrite growth, as well as neuroprotective processes.

Methylation of the distal promoter region of ALS2, particularly at CpG4, has been linked to suicides among individuals with a history of childhood ill-treatment. The CpG4 site overlaps with the binding region for upstream stimulating factor 1 (USF1), leading to the hypothesis that USF1 plays a role in ALS2 expression. Methylation at CpG4 is thought to inhibit USF1 activity, reducing ALS2 promoter activity and, consequently, the production of protein isoform 2 in the hippocampus[95].

Interestingly, reduced levels of ALS2 isoform 2 are observed in suicidal individuals regardless of ill-treatment history, but the methylation patterns differ between ill-treated and non-ill-treated individuals. This suggests that while methylation is associated with ill-

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[93] P.O. MCGOWAN, *et al. Epigenetic Regulation of the Glucocorticoid Receptor in Human Brain Associates with Childhood Abuse, Nat Neurosci*, 2009, 12, pp. 342-348.

[94] P.O. MCGOWAN, M.J. MEANEY, M. SZYF. *Diet and the epigenetic (re)programming of phenotypic differences in behavior*, Brain Res, 2008, 1237, pp. 12-24.

[95] B. LABONTÉ, *et al. Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder, Transl Psychiatry*, 2014, 4, pp. e368.

treatment, other epigenetic mechanisms, such as histone modifications, may account for reduced ALS2 expression in non-ill-treated suicides. The differential methylation observed between these groups indicates that the epigenetic changes are related to childhood ill-treatment and independent of suicide.

Collectively, these findings suggest that the genes discussed above, including ALS2, may contribute to the development of neuropsychiatric disorders in adulthood[96].

In studies examining whole-genome methylation profiling, several important findings have emerged. Naumova et al.[97] identified the involvement of genes associated with cell signaling regulation, while Smith et al. highlighted the role of genes linked to inflammation and immune responses[98].

A genome-wide DNA methylation study by Yang et al. observed significant differences in methylation patterns among children who experienced abuse compared to control groups[99]. Key findings include:

- Altered methylation at several CpG sites, with 20% of these changes occurring in intergenic regions. These regions are critical for gene regulation and the spatial organization of the genome, containing binding sites for transcription factors and chromatin insulators. Such alterations are thought to affect chromosomal interactions, influencing gene expression both locally and distally. Methylation changes in

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[96] J. NÖTHLING, cited above p. 55.

[97] O.Y. NAUMOVA, *et al. Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents, Dev Psychopathol*, 2012, 24, pp. 143-155.

[98] A.K. SMITH, *et al. Differential Immune System DNA Methylation and Cytokine Regulation in Post-Traumatic Stress Disorder, Am J Med Genet B Neuropsychiatr Genet*, 2011, 156, pp. 700-708.

[99] B.Z. YANG, *et al. Child abuse and epigenetic mechanisms of disease risk, Am J Prev Med*, 2013, 44, pp. 101-107.

intergenic regions have been associated with neuropsychiatric disorders, cardiovascular diseases, obesity, and various cancers.

- The majority of altered CpG sites were located in intragenic regions, with several genes showing significant methylation changes. These genes are implicated in critical biological processes, including cerebral cortex development (CCDCC85C), depression and substance abuse risk (PTPRN2), asthma susceptibility (FANK1), and cancer (WNT3A).

- Methylation changes were also observed in genes involved in biological pathways relevant to psychiatric disorders, substance abuse, cardiovascular diseases, stroke, respiratory diseases, diabetes, and cancer. This includes alterations in genes regulating neurogenesis, axonal development, blood vessel morphogenesis, interleukin regulation, leptin signaling, and cancer-related pathways such as WNT and NOTCH signaling. Furthermore, networks involved in inflammation (e.g., IL-2 signaling) and gene regulation (e.g., transcription initiation and telomere length regulation) were also found to be affected.

- In abused children, these methylation alterations were also observed in genes linked to several cancers, including lung, colorectal, prostate, breast, colon, and ovarian cancers. The increased cancer risk may be partly explained by the higher prevalence of risk behaviors (e.g., smoking, alcohol use) in adults who experienced childhood abuse, which are potentially driven by epigenetic modifications.

In a study by Mehta et al., comparison of whole-genome methylation profiles between PTSD patients with a history of childhood trauma and those without trauma revealed only a 2% overlap in methylation

patterns[100]. This suggests that childhood trauma may lead to profound epigenetic alterations, significantly reshaping the epigenome.

2.3. The relationship between sexual violence and post-traumatic stress disorder.

PTSD is characterized by a range of symptoms outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[101]. The criteria specified herein apply specifically to adolescents, adults, and children aged six years and older:

A. Exposure to actual or threatened death, serious injury, or sexual violence in one (or more) of the following ways:

1. Directly experiencing the traumatic event(s).

2. Witnessing, in person, the event(s) as it occurred to others.

3. Learning that the traumatic event(s) occurred to a close family member or close friend. In cases of actual or threatened death of a family member or friend, the event(s) must have been violent or accidental.

4. Experiencing repeated or extreme exposure to aversive details of the traumatic event(s) (e.g., first responders collecting human remains; police officers repeatedly exposed to details of child abuse). Note: Criterion A4 does not apply to exposure through electronic media, television, movies, or pictures, unless this exposure is work related.

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[100] D. MEHTA, *et al. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder, Proc Natl Acad Sci U S A*, 2013, 110, pp. 8302-8307.

[101] AMERICAN PSYCHIATRIC ASSOCIATION**.** *Diagnostic and statistical manual of mental disorders: DSM-5, 5th edn, American Psychiatric Publishing*, Washington, D.C., 2013, pp. 302-314.

B. Presence of one (or more) of the following intrusion symptoms associated with the traumatic event(s), beginning after the traumatic event(s) occurred:

1. Recurrent, involuntary, and intrusive distressing memories of the traumatic event(s). Note: In children older than 6 years, repetitive play may occur in which themes or aspects of the traumatic event(s) are expressed.

2. Recurrent distressing dreams in which the content and/or affect of the dream are related to the traumatic event(s). Note: In children, there may be frightening dreams without recognizable content.

3. Dissociative reactions (e.g., flashbacks) in which the individual feels or acts as if the traumatic event(s) were recurring. (Such reactions may occur on a continuum, with the most extreme expression being a complete loss of awareness of present surroundings.) Note: In children, trauma-specific reenactment may occur in play.

4. Intense or prolonged psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event(s).

5. Marked physiological reactions to internal or external cues that symbolize or resemble an aspect of the traumatic event(s).

C. Persistent avoidance of stimuli associated with the traumatic event(s), beginning after the traumatic event(s) occurred, as evidenced by one or both of the following:

1. Avoidance of or efforts to avoid distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s).

2. Avoidance of or efforts to avoid external reminders (people, places, conversations, activities, objects, situations) that arouse distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s).

D. Negative alterations in cognitions and mood associated with the traumatic event(s), beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the following:

1. Inability to remember an important aspect of the traumatic event(s) (typically due to dissociative amnesia, and not to other factors such as head injury, alcohol, or drugs).

2. Persistent and exaggerated negative beliefs or expectations about oneself, others, or the world (e.g., “I am bad,” “No one can be trusted,” “The world is completely dangerous,” “My whole nervous system is permanently ruined”).

3. Persistent, distorted cognitions about the cause or consequences of the traumatic event(s) that lead the individual to blame himself/herself or others.

4. Persistent negative emotional state (e.g., fear, horror, anger, guilt, or shame).

5. Markedly diminished interest or participation in significant activities.

6. Feelings of detachment or estrangement from others.

7. Persistent inability to experience positive emotions (e.g., inability to experience happiness, satisfaction, or loving feelings).

E. Marked alterations in arousal and reactivity associated with the traumatic event(s), beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the following:

1. Irritable behavior and angry outbursts (with little or no provocation), typically expressed as verbal or physical aggression toward people or objects.

2. Reckless or self-destructive behavior.

3. Hypervigilance.

4. Exaggerated startle response.

5. Problems with concentration.

6. Sleep disturbance (e.g., difficulty falling or staying asleep or restless sleep).

F. Duration of the disturbance (Criteria B, C, D and E) is more than 1 month.

G. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

H. The disturbance is not attributable to the physiological effects of a substance (e.g., medication, alcohol) or another medical condition.

Specify whether:

With dissociative symptoms: The individual's symptoms meet the criteria for posttraumatic stress disorder, and in addition, in response to the stressor, the individual experiences persistent or recurrent symptoms of either of the following:

1. Depersonalization: Persistent or recurrent experiences of feeling detached from, and as if one were an outside observer of, one's mental

processes or body (e.g., feeling as though one were in a dream; feeling a sense of unreality of self or body or of time moving slowly).

2. Derealization: Persistent or recurrent experiences of unreality of surroundings (e.g., the world around the individual is experienced as unreal, dreamlike, distant, or distorted). Note: To use this subtype, the dissociative symptoms must not be attributable to the physiological effects of a substance (e.g., blackouts, behavior during alcohol intoxication) or another medical condition (e.g., complex partial seizures).

Specify whether:

With delayed expression: If the full diagnostic criteria are not met until at least 6 months after the event (although the onset and expression of some symptoms may be immediate).

The prevalence of PTSD following experiences of violence is addressed inconsistently in scientific literature. For example, one study reports that 46% of women who have experienced rape develop PTSD over their lifetime[102], while another emphasizes that rape is the most common cause of PTSD in women[103]. In contrast, a more recent study estimates that PTSD develops in 23.6% of cases of violence against women[104]. This latter figure aligns closely with findings from a WHO survey conducted across 11 nations, which observed a 20.2% incidence of PTSD following violence[105].

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[102] M. CREAMER, P. BURGESS, A.C. MCFARLANE *Post-traumatic stress disorder: findings from the Australian National Survey of Mental Health and Well-being, Psychol Med*, 2001, 31, 7, pp. 1237-1247.

[103] N. BRESLAU, *et al. Vulnerability to assaultive violence: further specification of the sex difference in post-traumatic stress disorder, Psychol Med*, 1999, 29, 4, pp. 813-821.

[104] G. CARDOSO, *et al. Trauma exposure and PTSD in Portugal: findings from the World Mental Health Survey initiative, Psychiatry Res*, 2020, 284, pp. 112644.

[105] K.M. SCOTT, *et al. Post-traumatic stress disorder associated with sexual assault among women in the WHO World Mental Health Surveys, Psychol Med*, 2018, 48, 1, pp. 155-167.

PTSD has profound effects on major physiological systems, including the nervous, endocrine, and immune systems[106]. Central to the regulation of these systems is the hypothalamic-pituitary-adrenal (HPA) axis, which maintains homeostasis via a negative feedback mechanism. Cortisol, the primary stress hormone regulated by the HPA axis, is released in response to corticotropin-releasing hormone (CRH) and is inhibited through feedback at the hypothalamic and pituitary levels. Upon detection of environmental stress by the limbic system[107], neurons in the paraventricular nucleus of the hypothalamus are stimulated to produce CRH and arginine vasopressin (AVP). These hormones act on the pituitary gland to trigger the release of adrenocorticotropic hormone (ACTH), which subsequently stimulates the adrenal glands to release glucocorticoids. These glucocorticoids, including cortisol, circulate throughout the body, binding to glucocorticoid receptors (GRs) in various tissues, including the brain[108]. The activity of GRs is regulated by a complex array of proteins, such as heat shock proteins and FK506 binding proteins (FKBP), and their activation leads to the regulation of gene transcription related to glucocorticoid response. The tissue-specific nature of these responses allows for the rapid inactivation of genes involved in the HPA axis, contributing to the negative feedback process[109].

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[106] N. BRUNELLO, *et al. Posttraumatic stress disorder: diagnosis and epidemiology, comorbidity and social consequences, biology and treatment, Neuropsychobiology*, 2001, 43, 3, pp. 150-162.

[107] G.P. CHROUSOS, P.W. GOLD. *The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis*, JAMA, 1992, 267, 9, pp. 1244-1252.

[108] N.C. NICOLAIDES, *et al. The human glucocorticoid receptor: molecular basis of biologic function, Steroids,* 2010, 75, 1, pp. 1-12.

[109] G.M. RUSSELL, *et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males, J Neurosci*, 2010, 30, 17, pp. 6106-6115.

Severe psychological trauma, such as sexual abuse, can profoundly alter the body's stress response by elevating CRH levels and disrupting the regulation of the HPA axis[110]. This dysregulation manifests as a reduction in CRH receptors in the anterior pituitary, decreased responsiveness of the pituitary to CRH, and impaired negative feedback inhibition[111]. A diminished response to CRH can lead to hyperactivation of the HPA axis, further disrupting cortisol's inhibitory feedback mechanisms. As cortisol plays a critical role in regulating various neural systems, its dysregulation can affect the mesolimbic dopaminergic system, contributing to abnormal fear responses and persistent low-level depression[112].

It was initially hypothesized that PTSD development was linked primarily to the hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress, leading to elevated cortisol levels. However, subsequent studies have shown that cortisol levels in individuals with PTSD and controls are often similar, suggesting that the relationship between cortisol and PTSD is more complex[113]. This discrepancy may arise from the considerable variability in factors influencing cortisol production. Instead, a more consistent finding across studies is an increase in corticotropin-releasing factor (CRF)

[110] D.J. NUTT. *The psychobiology of posttraumatic stress disorder, J Clin Psychiatry*, 2000, 61, Suppl 5, pp. 24-32.

[111] Y. TIZABI, G. AGUILERA, G.M. GILAD. *Age-related reduction in pituitary corticotropin-releasing hormone receptors in two rat strains, Neurobiol Aging*, 1992, 13, 2, pp. 227-230.

[112] B.D. PERRY, *et al. Childhood trauma, the neurobiology of adaptation, and use-dependent development of the brain: how states become traits, Infant Mental Health Journal*, 1996, 16, 4, pp. 271-291.

[113] D.G. BAKER, *et al. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder, Am J Psychiatry*, 1999, 156, 4, pp. 585-588.

levels in both blood and cerebrospinal fluid (CSF)[114], along with hyperresponsiveness of glucocorticoid receptors (GR)[115].

A further consequence of HPA axis dysregulation is the disruption of sex-linked steroid hormones, such as estrogen and testosterone, which can contribute to reproductive issues, including infertility[116]. Beyond endocrine dysfunction, the disruption of adrenergic, glutamatergic, and serotonergic neurotransmitter systems plays a crucial role in the structural and functional brain abnormalities underlying PTSD symptoms. The persistent re-experiencing of traumatic events leads to sustained elevations in CRF, which has been linked to hippocampal damage[117]. This hippocampal damage is further evidenced by a reduction in hippocampal volume, which correlates with cognitive impairments and decreased overall performance[118].

Moreover, HPA axis dysregulation can stimulate the adrenergic system by activating gamma-aminobutyric acid (GABA) receptors, resulting in alterations in memory and perception. The intricate interplay between these systems may account for the strong connection between emotional processing and episodic memory. One hypothesis suggests that during PTSD flashbacks, traumatic memories are accompanied by heightened GABA receptor stimulation due to

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[114] J.D. BREMNER, E. VERMETTEN. *Stress and development: behavioral and biological consequences, Dev Psychopathol*, 2001, 13, 3, pp. 473-489.

[115] T. RINNE, *et al. Hyperresponsiveness of hypothalamic-pituitary-adrenal axis to combined dexamethasone/corticotropin-releasing hormone challenge in female borderline personality disorder subjects with a history of sustained childhood abuse, Biol Psychiatry*, 2002, 52, 11, pp. 1102-1112.

[116] C. RIVIER, J. RIVIER, W. VALE. *Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor, Science*, 1986, 231, 4738, pp. 607-609.

R. WAMSER-NANNEY. *Trauma exposure, PTSD and indices of fertility, J Psychosom Obstet Gynaecol*, 2020, 41, 2, pp. 116-121.

[117] E. VAN VOORHEES, A. SCARPA. *The effects of child maltreatment on the hypothalamic-pituitary-adrenal axis, Trauma Violence Abuse*, 2004, 5, 4, pp. 333-352.

[118] J.D. BREMNER, E. VERMETTEN. *Neuroanatomical changes associated with pharmacotherapy in posttraumatic stress disorder, Ann N Y Acad Sci*, 2004, 1032, pp. 154-157.

increased release of endogenous benzodiazepines[119]. Additionally, elevated catecholamine levels, a hallmark of the autonomic nervous system's "fight or flight" response, have been observed in PTSD patients[120], leading to exaggerated alarm responses. It has been postulated that excessive adrenergic activation during trauma may contribute to the consolidation of long-term traumatic memories in PTSD[121].

The immune system, which is regulated by both the nervous and endocrine systems, may also be significantly impacted by PTSD[122]. PTSD is frequently associated with various inflammatory conditions, likely due to the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which alters immune function by reducing the production of interleukins and other immune-related factors[123]. Notably, immune alterations differ between sexes: men with PTSD often experience suppression of cell-mediated immune responses, whereas women exhibit an increase in these responses[124]. Additionally, trauma-induced stress activates the immune system, leading to the peripheral release of cytokines and other immune molecules[125]. These immune factors can

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[119] J. DAVIDSON, *et al. Tribulin in post-traumatic stress disorder, Psychol Med*, 1988, 18, 4, pp. 833-836.

[120] H. ANISMAN. *Sensitization in relation to posttraumatic stress disorder, Biol Psychiatry*, 2011, 70, 5, pp. 404-405.

[121] R.K. PITMAN. *Post-traumatic stress disorder, hormones, and memory, Biol Psychiatry*, 1989, 26, 3, pp. 221-223.

[122] J.A. BOSCARINO. *Posttraumatic stress disorder and physical illness: results from clinical and epidemiologic studies*, *Ann N Y Acad Sci*, 2004, 1032, pp. 141-153.

[123] M.S. BREEN, *et al. Gene networks specific for innate immunity define post-traumatic stress disorder, Mol Psychiatry,* 2015, 20, 12, pp. 1538-1545.

[124] P. TUCKER, *et al. Neuroimmune and cortisol changes in selective serotonin reuptake inhibitor and placebo treatment of chronic posttraumatic stress disorder, Biol Psychiatry*, 2004, 56, 2, pp. 121-128.

[125] I.C. PASSOS, *et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression, Lancet Psychiatry*, 2015, 2, 11, pp. 1002-1012.

cross into the central nervous system (CNS), where they influence neural circuits involved in mood regulation and behavior[126].

Cognitive factors play a critical role in the onset, severity, and recovery from PTSD following sexual abuse. Key cognitive factors include feelings of mental defeat, confusion, negative appraisal of emotions and symptoms, avoidance behaviors, and perceptions of negative social responses[127]. For instance, if a rape victim perceives a lack of social support from others, the likelihood of developing PTSD significantly increases. Furthermore, victims may experience a sense of future helplessness, often accompanied by self-blame for the event or attribution of blame to others who failed to prevent the situation. These cognitive patterns can lead to more complex cases of PTSD that are challenging to treat[128].

The victim's perceived stress immediately following the traumatic event is also critical, as it can exacerbate anxiety and fear in the ensuing months. For example, forensic clinical examinations can be a significant source of stress for the victim, and as such, these evaluations should be conducted in a manner that minimizes additional trauma[129].

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[126] L. CAPURON, A.H. MILLER. *Immune system to brain signaling: neuropsychopharmacological implications, Pharmacol Ther*, 2011, 130, 2, pp. 226-238.

[127] E. DUNMORE. *Cognitive factors involved in the onset and maintenance of posttraumatic stress disorder (PTSD) after physical or sexual assault, Behav Res Ther*, 1999, 37, 9, pp. 809-829.

[128] P.A. FRAZIER. *Perceived control and distress following sexual assault: a longitudinal test of a new model, J Pers Soc Psychol*, 2003, 84, 6, pp. 1257-1269.

[129] H. RESNICK, *et al. Prevention of post-rape psychopathology: preliminary findings of a controlled acute rape treatment study, J Anxiety Disord*, 1999, 13, 4, pp. 359-370.

2.4. Epigenetic mechanisms underlying post-traumatic stress disorder.

Traumatic experiences, including gender-based violence, appear to influence genome regulation and expression through epigenetic modifications as a biological response to trauma[130]. Indeed, there is evidence supporting the role of epigenetic regulation in shaping both physiological and behavioral responses to environmental stressors.[131] The development of PTSD has been investigated at both the genomic[132] and epigenomic levels. While PTSD has a clear genetic component, with heritability estimates ranging from 24% to 72%, its genetic mechanisms remain largely unidentified, with most implicated genes found through Genome-Wide Association Studies (GWAS)[133].

The relationship between PTSD and epigenetic modifications has been explored in various populations, including war veterans, abused children, and Holocaust survivors[134].

Stressors leading to PTSD are among the environmental stimuli capable of altering DNA methylation patterns in the brain, particularly affecting genes involved in regulating the hypothalamic-pituitary-

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[130] A.K.S. CONCHING, Z. THAYER. *Biological pathways for historical trauma to affect health: A conceptual model focusing on epigenetic modifications,* Soc Sci Med, 2019, 230, pp. 74-82.

[131] L. SIPAHI, *et al.* Ancient evolutionary origins of epigenetic regulation associated with posttraumatic stress disorder, *Front Hum Neurosci,* 2014, 8, p. 284.; N. CHMIELEWSKA, *et al. Epigenetic mechanisms of stress and depression,* Psychiatr Polska, 2019, 53, pp. 1413-1428.

[132] K.L. CHAN, *et al.* The association between intimate partner violence against women and newborn telomere length, *Transl Psychiatry*, 2019, 9, p. 239; C.M NIEVERGELT, *et al. Genomic predictors of combat stress vulnerability and resilience in U.S. Marines: A genome-wide association study across multiple ancestries implicates PRTFDC1 as a potential PTSD gene,* Psychoneuroendocrinology, 2015, 51, pp. 459-471.

[133] M.B. STEIN. *et al. Genome-wide association analyses of post-traumatic stress disorder and its symptom subdomains in the Million Veteran Program, Nat Genet,* 2021, 53, pp. 174-184.

[134] R. YEHUDA, *et al. Influences of Maternal and Paternal PTSD on Epigenetic Regulation of the Glucocorticoid Receptor Gene in Holocaust Survivor Offspring, Am J Psychiatry*, 2014, 171, pp. 872-880; M.S. TRIVEDI, *et al. Alterations in DNA Methylation Status Associated with Gulf War Illness, DNA Cell Biol*, 2019, 38, pp. 561-571.

adrenal (HPA) axis. Epigenetic modifications linked to HPA axis regulation have been found not only in neuronal cells but also in peripheral tissues such as blood and mucous membranes[135]. Given the similarities between epigenetic patterns in nervous system cells and peripheral tissue cells, ongoing research is focused on understanding these relationships[136]. Venous blood, being more accessible than brain tissue, offers a valuable substrate for studying epigenetic regulation, though findings from blood samples should ideally be corroborated by future studies in brain tissue[137].

Maintaining homeostasis of the HPA axis is critical, as its dysregulation is closely associated with psychiatric disorders, including PTSD[138]. Excessive suppression of the HPA axis has been linked to traumatic events and PTSD[139]. This suppression is regulated by the glucocorticoid receptor (GR), encoded by the NR3C1 gene, which mediates the negative feedback loop of the HPA axis. A study by McGowan et al. demonstrated that methylation of the NR3C1 promoter is associated with childhood ill-treatment, revealing that individuals who experienced childhood abuse and later died by suicide exhibited greater methylation of the NR3C1 promoter and reduced GR mRNA expression in the hippocampus compared to controls[140].

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[135] A.S. ZANNAS, A.E. WEST. *Epigenetics and the regulation of stress vulnerability and resilience*, *Neuroscience*, 2014, 264, pp. 157-170.

[136] R.S. LEE, A. SAWA. *Environmental stressors and epigenetic control of the hypothalamic-pituitary-adrenal axis*, *Neuroendocrinology*, 2014, 100, 4, pp. 278-287.

[137] K.M. BAKULSKI, *et al. Epigenetic Research in Neuropsychiatric Disorders: the "Tissue Issue"*, *Curr Behav Neurosci Rep*, 2016, 3, 3, pp. 264-274.

[138] K. GUDSNUK, F.A. CHAMPAGNE. *Epigenetic Influence of Stress and the Social Environment*, *ILAR J*, 2012, 53, pp. 279-288.

[139] M.C. MORRIS, B.E. COMPAS, J. GARBER. *Relations among posttraumatic stress disorder, comorbid major depression, and hpa function: a systematic review and meta-analysis, Clin Psychol Rev*, 2012, 32, pp. 301-315.

[140] P.O. MCGOWAN, cited above, p. 57.

Conversely, other studies have shown decreased methylation levels in the NR3C1 promoter regions in individuals with PTSD compared to those without the disorder[141]. Notably, methylation of the exon 1F promoter region of NR3C1 appears to be inversely correlated with PTSD symptom severity, suggesting that therapeutic interventions could aim to increase methylation in these regions[142]. Hyper-methylation of the NR3C1 promoter has been found to inversely correlate with lifetime PTSD risk[143].

One study on genocide survivors in Rwanda found that decreased methylation of the exon 1F promoter of the NR3C1 gene correlated with the persistence of traumatic memories and heightened PTSD risk, particularly in male subjects. Interestingly, this effect was not observed in female subjects, possibly suggesting that glucocorticoids have less influence on memory formation in women, though the underlying reasons for this remain unclear[144].

These differences in methylation are localized to the binding site of the transcription factor NGFI-A, where methylation levels are negatively correlated with NR3C1 expression, with hypermethylation being associated with depressive symptoms. However, some research has found no statistically significant differences in NR3C1 methylation between trauma-exposed individuals and healthy controls, leading to

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[141] H. PALMA-GUDIEL, *et al. Glucocorticoid Receptor Gene (NR3C1) Methylation Processes as Mediators of Early Adversity in Stress-Related Disorders Causality: A Critical Review, Neurosci Biobehav Rev*, 2015, 55, pp. 520-535.

[142] R. YEHUDA, *et al. Epigenetic Biomarkers as Predictors and Correlates of Symptom Improvement Following Psychotherapy in Combat Veterans with PTSD, Front Psychiatry*, 2013, 4, pp. 118.

[143] R. YEHUDA, cited above, p. 70.

[144] V. VUKOJEVIC, *et al. Epigenetic Modification of the Glucocorticoid Receptor Gene is Linked to Traumatic Memory and Post-Traumatic Stress Disorder Risk in Genocide Survivors, J Neurosci*, 2014, 34, pp. 10274-10284.

the hypothesis that methylation may be a pre-existing vulnerability rather than a direct consequence of PTSD. This methylation pattern could potentially develop during childhood in response to early environmental stressors.

NR3C1-1F hypermethylation has been proposed as a potential protective factor, as it is associated with reduced memory consolidation of traumatic events. However, other studies have presented mixed findings, showing that increased methylation of the NR3C1-1F region is correlated with worsening mental health outcomes[145]. These divergent results highlight the complexity of the epigenetic regulation in PTSD, suggesting that further research is needed to fully understand the underlying mechanisms.

The FKBP51 gene encodes the FK506-binding protein 51, a co-chaperone of the glucocorticoid receptor (GR) that plays a critical role in modulating GR sensitivity by forming an intracellular negative feedback loop[146]. FKBP51 expression is induced by glucocorticoids and negatively regulates GR activity, promoting glucocorticoid resistance. This increased FKBP51 activity raises the set-point of the hypothalamic-pituitary-adrenal (HPA) axis and delays the system's return to homeostasis following a stressor.

Children who experience abuse are more likely to develop PTSD if they possess certain polymorphisms in the FKBP5 gene, which encodes

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[145] R.R. SCHÜR, *et al. Longitudinal Changes in Glucocorticoid Receptor Exon 1F Methylation and Psychopathology After Military Deployment, Transl Psychiatry*, 2017, 7, pp. e1181; N. PERROUD, *et al. Response to Psychotherapy in Borderline Personality Disorder and Methylation Status of the BDNF Gene, Transl Psychiatry*, 2013, 3, pp. e207.

[146] G.M. WOCHNIK, *et al. FK506-Binding Proteins 51 and 52 Differentially Regulate Dynein Interaction and Nuclear Translocation of the Glucocorticoid Receptor in Mammalian Cells, J Biol Chem*, 2005, 280, pp. 4609-4616.

a chaperone for the glucocorticoid receptor[147]. These polymorphisms are associated with reduced methylation in the intronic regions of the gene, resulting in elevated FKBP5 expression[148]. FKBP5 expression may also be modulated by stress-related molecules with epigenetic activity, and it has been suggested that FKBP5 methylation levels in peripheral blood could serve as potential biomarkers for stress exposure[149].

Additionally, other studies have examined the regulation of the ADCYAP1 gene, which encodes pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide involved in stress response and fear regulation. Methylation of the gene encoding the PACAP receptor (ADCYAP1R1) has been associated with PTSD symptoms in both men and women, further implicating this pathway in the pathophysiology of PTSD[150].

The SKA2 gene encodes a protein that plays a critical role in the transactivation of the glucocorticoid receptor[151]. Elevated methylation of SKA2 has been linked to posttraumatic stress disorder (PTSD) symptoms[152]. Specifically, methylation at the SKA2 cg13989295 locus

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[147] E.B. BINDER. *The Role of FKBP5, a Co-Chaperone of the Glucocorticoid Receptor in the Pathogenesis and Therapy of Affective and Anxiety Disorders, Psychoneuroendocrinology*, 2009, 34, pp. S186-S195.

[148] T. KLENGEL, *et al. Allele-Specific FKBP5 DNA Demethylation Mediates Gene-Childhood Trauma Interactions, Nat Neurosci*, 2013, 16, pp. 33-41.

[149] A.S. ZANNAS, cited above, p. 71; M.A. ARGENTIERI, *et al. Epigenetic Pathways in Human Disease: The Impact of DNA Methylation on Stress-Related Pathogenesis and Current Challenges in Biomarker Development, eBioMedicine*, 2017, 18, pp. 327-350; A. CHATZITTOFIS, *et al. HPA Axis Dysregulation is Associated with Differential Methylation of CpG-Sites in Related Genes*, Sci Rep, 2021, 11, pp. 20134.

[150] L.M. ALMLI, *et al.* ADCYAP1R1 *Genotype Associates with Post-Traumatic Stress Symptoms in Highly Traumatized African-American Females, Am J Med Genet B Neuropsychiatr Genet*, 2013, 162, pp. 262-272.

[151] L. RICE, *et al. Identification and Functional Analysis of SKA2 Interaction with the Glucocorticoid Receptor, J Endocrinol*, 2008, 198, pp. 499-509.

[152] M.P. BOKS, cited above, p. 56.

has been associated with bilateral reductions in cortical thickness in the frontal regions of the brain, correlating with worsening PTSD symptomatology[153].

Trauma exposure and PTSD may also be associated with epigenetic modifications in genes involved in inflammatory and immune responses. One widely studied biomarker of peripheral inflammation, C-reactive protein (CRP), has been implicated in PTSD in several recent studies[154]. In a study of veterans with high rates of PTSD, Miller and colleagues observed that PTSD severity was positively correlated with CRP levels and inversely correlated with DNA methylation at cg10636246 in the Absent in Melanoma 2 (AIM2) gene, a receptor involved in innate immune response[155]. The findings suggested that the association between PTSD severity and elevated CRP levels was mediated by methylation at this locus, indicating that PTSD might be linked to an increase in inflammatory markers through AIM2-associated methylation changes.

Additionally, a longitudinal study of U.S. military personnel found increased serum methylation levels of the gene encoding interleukin 18 (IL18) and its corresponding lncRNA transcript in individuals who later developed PTSD[156].

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[153] N. SADEH, *et al. SKA2 Methylation is Associated with Decreased Prefrontal Cortical Thickness and Greater PTSD Severity Among Trauma-Exposed Veterans, Mol Psychiatry*, 2016, 21, pp. 357-363.

[154] C. LIN, *et al. Affect, Inflammation, and Health in Urban At-Risk Civilians, J Psychiatr Res*, 2018, 104, pp. 24-31; A. O'DONOVAN, *et al. Current Posttraumatic Stress Disorder and Exaggerated Threat Sensitivity Associated with Elevated Inflammation in the Mind Your Heart Study, Brain Behav Immun*, 2017, 60, pp. 198-205.

[155] M.W. MILLER, *et al. CRP Polymorphisms and DNA Methylation of the AIM2 Gene Influence Associations Between Trauma Exposure, PTSD, and C-Reactive Protein, Brain Behav Immun*, 2018, 67, pp. 194-202.

[156] J.A. RUSIECKI, *et al. PTSD and DNA Methylation in Select Immune Function Gene Promoter Regions: A Repeated Measures Case-Control Study of U.S. Military Service Members, Front Psychiatry*, 2013, 4, pp. 56.

In another study, hypomethylation of the SLC6A4 gene, which encodes the serotonin transporter, was identified as a potential risk factor for developing PTSD following trauma exposure[157]. Conversely, hypermethylation of SLC6A4 may serve as a protective factor. Although methylation of this gene is not directly implicated in the pathogenesis of PTSD, it is considered a marker of vulnerability or resilience.

Using an epigenome-wide association study (EWAS) approach, Uddin and colleagues found that individuals with PTSD exhibited greater demethylation of genes associated with immune and inflammatory functions in peripheral blood cells compared to non-PTSD controls. Furthermore, changes in genes related to sensory perception of sound, such as OTOF, were also noted, potentially explaining the heightened response to auditory stimuli and the hyperarousal state characteristic of PTSD[158].

In a study by Smith et al., differential methylation of CpG sites was observed in five genes— toll-like receptor 8 (TLR8), annexin A2 (ANXA2), acid phosphatase 5, tartrate resistant (ACP5), translocated promoter region (TPR), and C-type lectin domain family 9, member A (CLEC9A)—among individuals with PTSD compared to controls[159]. Notably, methylation of a CpG site near the neuropeptide receptor FF 2 (NPFFR2) was inversely correlated with Total Life Stress (TLS) scores.

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[157] K.C. KOENEN, *et al. SLC6A4 Methylation Modifies the Effect of the Number of Traumatic Events on Risk for Posttraumatic Stress Disorder, Depress Anxiety*, 2011, 28, pp. 639-647.

[158] M. UDDIN, *et al. Epigenetic and Immune Function Profiles Associated with Posttraumatic Stress Disorder, Proc Natl Acad Sci U S A*, 2010, 107, pp. 9470-9475.

[159] A.K. SMITH, cited above, p. 58.

Genes such as TLR8, NPFFR2, and ACP5 are implicated in inflammatory pathways[160].

In a separate study involving Australian Vietnam War veterans, Mehta et al. identified significant associations between CpG methylation and PTSD symptom severity at sites within the genes encoding nerve growth factor (NGF), BR serine/threonine kinase 1 (BRSK1), lipocalin 8 (LCN8), and dedicator of cytokinesis 2 (DOCK2)[161]. Notably, DOCK2, previously linked to Alzheimer's disease and neuroinflammation[162], is expressed in the brain, immune cells[163], and some cancer cells[164].

In an epigenome-wide association study (EWAS) of veterans with PTSD, Logue et al. identified increased methylation of G0/G1 Switch 2 (G0S2) and hypomethylation of the Aryl-hydrocarbon Receptor Repressor (AHRR)[165].

Regarding the role of circulating miRNAs in PTSD, early studies focusing on combat veterans have reported elevated levels of miR-19a, miR-128, miR-15b, and miR-125b in individuals with PTSD. miR-19b has been implicated in the regulation of genes associated with fear

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[160] Y. MA, *et al. TLR8: An Innate Immune Receptor in Brain, Neurons and Axons, Cell Cycle*, 2007, 6, pp. 2859-2868; J. LAMEH, *et al. Neuropeptide FF Receptors Have Opposing Modulatory Effects on Nociception, J Pharmacol Exp Ther*, 2010, 334, pp. 244-254; M.S. KIM, *et al. MCP-1-Induced Human Osteoclast-Like Cells Are Tartrate-Resistant Acid Phosphatase, NFATc1, and Calcitonin Receptor-Positive But Require Receptor Activator of NFkappaB Ligand for Bone Resorption, J Biol Chem*, 2006, 281, pp. 1274-1285.

[161] D. MEHTA, *et al. Genomewide DNA Methylation Analysis in Combat Veterans Reveals a Novel Locus for PTSD, Acta Psychiatr Scand*, 2017, 136, pp. 493-505.

[162] P.J. CIMINO, *et al. DOCK2 is a Microglial Specific Regulator of Central Nervous System Innate Immunity Found in Normal and Alzheimer's Disease Brain, Am J Pathol*, 2009, 175, pp. 1622-1630.

[163] A. NISHIKIMI, *et al. Immune Regulatory Functions of DOCK Family Proteins in Health and Disease, Exp Cell Res*, 2013, 319, pp. 2343-2349.

[164] Y. CHEN, *et al. Dock2 in the Development of Inflammation and Cancer, Eur J Immunol*, 2018, 48, pp. 915-922.

[165] M.W. LOGUE, *et al. An Epigenome-Wide Association Study of Posttraumatic Stress Disorder in US Veterans Implicates Several New DNA Methylation Loci, Clin Epigenetics*, 2020, 12, pp. 46.

memory, suggesting that its dysregulation may contribute to the vulnerability, onset, or persistence of PTSD-like symptoms. Similarly, increased expression of miR-128, a brain-specific miRNA, is critical in reliving fear-associated symptoms and may play a role in the onset of PTSD symptomatology. miR-15b is involved in several key cellular processes such as cell division, metabolism, stress response, and angiogenesis. Its overexpression in PTSD has been linked to a higher risk of cancer and Alzheimer's disease, indicating that it likely contributes to both psychological and physical health impairments. miR-125b is involved in modulating immune responses, including the activation and regulation of the NFkB pathway, which is essential for immune function[166].

The current body of research on PTSD-related epigenetic modifications largely focuses on populations that are distantly related to the issue of violence against women. Studies involving female war veterans, the closest parallel, demonstrate that violence can induce molecular epigenetic changes, contributing to mental, physical, and chronic diseases[167]. However, there is a notable gap in research examining the relationship between IPV, PTSD, and epigenetic changes in women, underscoring the need for more longitudinal studies. One of the few studies addressing this issue is that of Nöthling et al.[168], which

[166] C.G. MARTIN, *et al. Circulating miRNA Associated with Posttraumatic Stress Disorder in a Cohort of Military Combat Veterans, Psychiatry Res*, 2017, 251, pp. 261-265.

[167] M.R. GERBER, *et al. Women Veterans and Intimate Partner Violence: Current State of Knowledge and Future Directions, J Women's Health*, 2014, 23, pp. 302-309; E.M. SCHMIDT, *et al. Four Decades After War: Incident Diabetes Among Women Vietnam-Era Veterans in the HealthViEWS Study, Women's Health Issues*, 2019, 29, pp. 471-479.

[168] J. NÖTHLING, *et al. Genome-Wide Differentially Methylated Genes Associated with Posttraumatic Stress Disorder and Longitudinal Change in Methylation in Rape Survivors, Transl Psychiatry*, 2021, 11, pp. 594.

identified associations between ADCYAP1 and BRSK2 in female survivors of sexual violence. The demethylation of BRSK2 may lead to impaired neuronal polarization, disrupted synaptic development, abnormal vesicle formation, and altered neurotransmission in individuals with PTSD. However, these findings are preliminary and require further investigation to confirm their significance.

Given its potential to reveal how environmental factors shape disease risk, epigenetics warrants broader investigation in this context[169].

[169] L. ZHANG, Q. LU, C. CHANG. *Epigenetics in Health and Disease, in C. CHANG, Q. LU. (eds.) Epigenetics in Allergy and Autoimmunity, Singapore*, 2020, 1253, pp. 3-55.

Chapter III
THE PRELIMINARY STUDY OF THE EPIGENETICS FOR WOMEN (EPIWE) PROJECT

Contents: 3.1 Aim of the study. 3.2 Materials and Methods. 3.3 Results. 3.4 Discussion. 3.5 Study Limitations. 3.6 Conclusions.

3.1. Aim of the study.

The scientific literature reviewed highlights a significant gap in our understanding of the correlations between violence against women, PTSD, and epigenetic modifications. Specifically, there is a notable absence of comprehensive studies examining the prevalence of PTSD following episodes of sexual violence and IPV. While there is some research suggesting that sexual violence and IPV may induce changes in the epigenome—similar to the trauma-related epigenetic alterations observed in war veterans—rigorous investigations into this phenomenon in female victims of violence remain scarce.

Despite this lack of unified research, it is plausible that trauma from sexual violence and IPV induces epigenetic changes, such as DNA methylation, which could either contribute to the development of or foster resilience against PTSD. Epigenetic "scarring" in response to trauma is an emerging area of interest, but its specific role in women exposed to violence has yet to be thoroughly explored.

This thesis seeks to make an initial contribution to filling this gap by investigating potential epigenomic alterations triggered by violence. The study will employ a dual approach, combining the collection of

blood samples at intervals following the traumatic event with the administration of detailed anamnestic and clinical questionnaires. By focusing on women who have experienced violence, the study aims to assess the prevalence of PTSD and to identify specific epigenetic modifications that may serve as biological markers for clinical evaluations of susceptibility to PTSD.

A key component of the research involves identifying candidate genes associated with PTSD, as determined through an exhaustive review of current literature. The subsequent epigenetic profiling will center on DNA methylation, a well-established mechanism of gene regulation that has been implicated in stress-related disorders. The study's novel aspect lies in its long-term scope: through an 18-month follow-up with the participants, the persistence or regression of these epigenetic alterations will be monitored in relation to therapeutic interventions, such as psychotherapy. This longitudinal aspect will allow for an exploration of the potential reversibility of epigenetic changes and their relation to trauma recovery, offering important insights into personalized therapeutic approaches for female victims of violence.

This research not only aims to enhance our understanding of PTSD in the context of sexual violence and IPV but also seeks to advance the clinical application of epigenetic markers in the diagnosis and treatment of trauma-related disorders.

This pilot study represents a preliminary phase of the broader EpiWe (Epigenetics for WomEn) project, which is designed to investigate epigenetic signatures associated with PTSD in the context of violence

against women. The overarching goal of the EpiWe project is to expand the cohort size in order to more comprehensively identify and characterize epigenetic markers that may be linked to PTSD resulting from violence against women.

3.2. Materials and Methods.

The study population consisted of female victims of violence within intimate or relational contexts, who sought emergency care at two participating centers: SVSeD at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan and the Ospedale San Paolo in Naples. Prior to initiating the study, approval was obtained from the relevant ethics committees, including the Institutional Review Board (IRB). Specifically, clearance was granted by the Ethical Committee of the Italian National Institute of Health (Prot 23/07/2015-0022568) and the Ethical Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Prot 18/05/2016-258_2016 bis, Milan, Italy).

The inclusion criteria for participants were as follows: women aged 16 years or older who presented to the emergency department with a confirmed diagnosis of violence, including sexual assault and/or physical abuse, and who provided informed consent. Due to the relatively low number of individuals accessing these services and the high dropout rates commonly observed in such populations, no specific exclusion criteria were applied.

Considering the sensitive and often traumatic circumstances under which victims of violence access the Anti-Rape Center, the implementation of a standard informed consent protocol was deemed

impractical. The urgency of the situation and the emotional distress experienced by the women made it inappropriate to overwhelm them with extensive study-related information at their initial emergency visit. In compliance with the European General Data Protection Regulation (GDPR) No. 679/2016, an alternative consent procedure was developed to ensure both ethical compliance and respect for the participants' well-being.

Under this modified protocol, at the time of their initial visit, participants were only required to provide consent for the collection and storage of biological samples. This limited scope of consent helped to avoid adding further emotional or cognitive burden during their critical moment of need. Detailed information regarding the study, including full informed consent, was deferred and provided to the participants during a follow-up visit approximately 3 to 4 days after their initial emergency department visit. At that time, the women were invited to participate in a psychological assessment and were presented with comprehensive details about the study. This approach ensured that participants were in a more stable emotional state, enabling them to make an informed and autonomous decision regarding their involvement in the study.

Importantly, participants who chose not to continue with the study had any previously collected biological samples destroyed, in line with the protocol. This procedure was carefully designed to protect the participants' privacy and autonomy while adhering to the ethical standards required for conducting research on vulnerable populations, such as women victims of violence.

The data used in this study were collected during the initial visit of women subjected to violence to the participating centers. These data were gathered through a series of comprehensive assessments conducted by both gynecologists and forensic medical professionals, as well as psychological evaluations performed by psychologists and social workers. The medical examinations included both general and gynecological objective assessments, while the psychological screening aimed to identify the presence of psychological disorders such as PTSD, anxiety, and depression. These assessments were conducted using structured interviews based on standardized protocols, including the Sexual Violence Relief guidelines[170].

The collected information encompasses various aspects of the patient's medical and psychological history, including physiological and pathological anamnesis, as well as proximate and remote health history. Additionally, details regarding the circumstances of the violence, the presence of any vulnerability factors in the victim, the characteristics of the assault, and information on the perpetrator were recorded. Clinical data from the physical and gynecological examinations were also documented, alongside assessments of the psychological impact of the violence and the patient's anthropometric measurements (e.g., weight and height). Clinical-laboratory data were obtained from medical records, including blood tests for screening infectious diseases, as well as toxicological, forensic, biological, and genetic analyses.

[170] Sexual Violence Relief, GUIDELINES health, medico-legal, psycho-social assistance in situations of violence against women and children, 2006, http://www.umanamenteonline.it/wp-content/uploads/2012/01/lineeguidasvs1.pdf (20/10/2024).

At the time of the initial first aid intervention, venous blood samples were collected from the participants, consisting of three 6ml K2EDTA blood tubes. These samples were essential for subsequent biological analyses.

During the psychological evaluation, participants were asked to complete four validated questionnaires, which were translated into Italian and aimed at assessing psychological well-being and identifying risk factors related to family and social contexts. These questionnaires included the following: the PTSD CheckList Civilian Version (PCL-C)[171] to assess symptoms related to PTSD, the Center for Epidemiologic Studies Depression Scale (CESD-R)[172] for evaluating depressive symptoms, the Medical Outcome Short Form Survey 12 (SF-12)[173] for measuring general quality of life, and the Spousal Assault Risk Assessment Guide - Screening (SARA-S) for assessing the risk of future violence recurrence[174]. The PCL-C, in conjunction with the diagnostic criteria outlined in the DSM-5, was specifically used to assess the prevalence of PTSD among female victims of violence.

For the purpose of calculating PTSD prevalence, all available data from women who accessed the SVSeD in 2019 were utilized, regardless of whether the participants subsequently agreed to take part in the epigenomic analysis. The year 2019 was selected as the reference

[171] F.W. WEATHERS, et al, The PTSD Checklist for DSM-5 (PCL-5), 2013, https://www.ptsd.va.gov/professional/assessment/adult-sr/ptsd-checklist.asp (20/10/2024).

[172] N.T. VAN DAM, M. EARLEYWINE. *Validation of the Center for Epidemiologic Studies Depression Scale--Revised (CESD-R): pragmatic depression assessment in the general population, Psychiatry Res*, 2011, 186, pp. 128-132.

[173] G. KODRALIU, *et al. Subjective health status assessment: evaluation of the Italian version of the SF-12 Health Survey. Results from the MiOS Project, J Epidemiol Biostat*, 2001, 6, pp. 305-316.

[174] P.R. KROPP. *Some Questions Regarding Spousal Assault Risk Assessment, Violence Against Women,* 2004, 10, pp. 676-697.

period to ensure the most reliable and representative dataset. The COVID-19 pandemic, which began in 2020, significantly altered the patterns[175] of access to anti-violence centers and changed the nature of violence against women, leading to an increase in intimate partner violence and a reduction in overall visits, with a shift towards telephone-based assistance. Therefore, given the significant disruption caused by the pandemic, the year 2019 was chosen as the baseline to reflect more stable pre-pandemic conditions, allowing for a clearer analysis of PTSD prevalence among female victims of violence during that time.

The assessment of each subject's physical health status was conducted using standardized trauma severity indicators, specifically the Abbreviated Injury Scale (AIS) and the Injury Severity Score (ISS)[176]. These tools provided a quantifiable measure of the physical impact of the violence sustained. In addition to these trauma scores, a thorough physical examination was performed to identify chronic illnesses commonly associated with exposure to violence. Conditions such as chronic pain syndrome, fibromyalgia, irritable bowel syndrome, gastrointestinal disorders, gynecological disorders, and sexual dysfunction were evaluated, as these are frequently observed in victims of long-term violence and trauma.

To capture both short-term and longer-term effects of violence on health and psychological well-being, the cohort of exposed patients was

[175] R. MAJEED-ARISS, cited above, p. 21; G. BARBARA, cited above, p. 21; A. VIERO, cited above, p. 21.

[176] L. GREENSPAN, B.A. MCLELLAN, H. GREIG. *Abbreviated Injury Scale and Injury Severity Score: a scoring chart, J Trauma,* 1985, 25, pp. 60-64.

subjected to a structured follow-up over an 18-month period. Follow-up assessments were conducted at 6-month, 12-month, and 18-month intervals, during which time the same protocols as those used during the initial evaluation were re-administered. This included medical and psychological examinations to track both physiological and psychological recovery or deterioration over time. If a participant failed to attend one of the follow-up appointments, all previously collected data and biological samples were still included in the study. This approach was adopted in response to the common occurrence of high dropout rates among patients followed by anti-violence centers. Given the nature of the population and the difficulties in maintaining continuous engagement, it was necessary to utilize all available samples to ensure that the study yielded a robust dataset and reliable results. Restricting the analysis to only those patients who attended all follow-ups would have significantly reduced the sample size, potentially leading to biased or underpowered findings. By including all available data, the study aimed to ensure a broader and more consistent analysis of the long-term health effects of violence.

For comparison purposes, a control group was recruited to evaluate the epigenomic findings in relation to a non-exposed population. The inclusion criteria for this group were as follows: female subjects aged 16 years or older, with no history of violence or trauma, and without any significant pathologies or medical conditions. These individuals were recruited from the Transfusion Centre of the Policlinico di Milano (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico) and were required to provide informed consent before participating. To

ensure comparability, an effort was made to match the control group to the exposed population in terms of age and ethnicity, allowing for more meaningful comparisons of the epigenomic results. The control group participants were administered the same set of psychological and health-related questionnaires described earlier, and a blood sample was collected following the same procedures used with the violence-exposed cohort.

This methodology ensures that the study maintains scientific rigor by incorporating a well-matched comparison group and allowing for a longitudinal analysis of both physiological and psychological effects in victims of violence. The inclusion of standardized trauma severity indicators and repeated follow-up assessments across an extended time frame contributes to a more comprehensive understanding of the lasting impacts of violence on women's health, and the role of epigenomic changes in shaping those outcomes.

The epigenomic analyses were conducted at the Molecular Pathology laboratories of the Pathology Unit at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. The target methylation sites for the candidate genes under investigation were selected based on existing literature, as they had been previously identified as associated with trauma and stress-related disorders. The specific genes analyzed include ADCYAP1, BDNF, CRHR1, DRD2, FKBP5, IGF2, LSD1, NR3C1, PRTFDC1, and SLC6A4. These genes are generally characterized by hypomethylation, except for IGF2, which is known to be approximately 50% methylated due to its imprinted nature[177].

[177] D. MEHTA, cited above, p. 77; N. PERROUD, *et al. The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis, World J Biol Psychiatry,* 2014,

DNA was extracted from the blood samples of both the victims of violence and the control group using the QIAsymphony® DNA Kit, following the procedures outlined in the QIAsymphony® DNA Handbook[178]. This allowed for high-quality, efficient DNA extraction, which is critical for ensuring the reliability of subsequent epigenomic analyses.

The extracted DNA was treated using the EZ DNA Methylation-Gold™ Kit (EZ DNA Methylation-Gold™ Kit Handbook[179]), which facilitates the conversion of unmethylated cytosines into uracils through a sodium bisulphite reaction, followed by sample purification. During amplification, these uracils are further converted into thymines, while methylated cytosines remain unchanged. This chemical transformation is essential for distinguishing between methylated and unmethylated cytosines in subsequent analyses.

To focus the study on specific regions of interest, the promoter regions of the selected genes were identified using a computational tool (FirstEF[180]) that simulates promoter sequences. Primers for these regions were then designed using EpiDesigner software[181], ensuring

15, pp. 334-345; J.A. RUSIECKI, *et al. DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members, Epigenomics*, 2012, 4, pp. 29-40; M. MIOZZO, V. VAIRA, S.M. SIRCHIA. *Epigenetic alterations in cancer and personalized cancer treatment, Future Oncol,* 2015, 11, pp. 333-348.

[178] Qiagen, QIAsymphony® DNA Handbook, 2010, https://www.qiagen.com/us/resources/download.aspx?id=f188cf11-c0b0-4c13-8687-f383d98bb986&lang=en (20/10/2024).

[179] The Epigenetics Company, EZ DNA Methylation-Gold™ Kit Handbook, 2021, https://files.zymoresearch.com/protocols/_d5005_d5006_ez_dna_methylation-goldga_o_kit.pdf (20/10/2024).

[180] FirstEF: First-Exon and Promoter Prediction Program for Human DNA, 2007, http://rulai.cshl.org/tools/FirstEF/ (20/10/2024).

[181] Agena Bioscience, EpiDesigner, 2022, https://www.epidesigner.com (20/10/2024).

precision in targeting the areas most relevant to gene expression and methylation regulation[182].

Gene	Chromosome [1]	Sequence ID[2]	start – end[3]	N° CpG sites[4]	CpG coverage[5]	PCR Product Size (bps) [6]
ADCYAP1	18	AP000894.6	110,108 - 110,333	10	9	226
BDNF	11	NG_011794.1	4,002 - 4,350	22	21	349
CRHR1	17	NG_009902.1	3,971 - 4,263	24	21	291
DRD2	11	NG_008841.1	4,581 - 4,789	12	12	209
FKBP5	6	NG_012645.2	35,687,781 – 35,688,046	15	10	266
IGF2	11	NG_008849.1	21,346 - 21,832	29	25	487
LSD1/KDM1A	1	NG_047129.1	5,217 - 5,464	25	18	248
NR3C1	5	NG_009062.1	36,173 - 36,575	47	27	403
PRTFDC1	10	NC_000010.11	24,953,017 – 24,953,295	6	4	279
SLC6A4	17	NG_011747.2	4,906 - 5,202	29	20	297

PCR Primer Table. Legend: 1. Chromosomal locus, 2. Reference sequence, 3. Genomic position, 4. CpG sites within the analyzed region, 5. CpG sites quantified using the EpiTYPER software, and 6. Amplicon length of the targeted loci.

The next phase involved the amplification of the selected DNA sequences using Polymerase Chain Reaction (PCR). The amplified

[182] L. FONTANA, *et al. Characterization of multi-locus imprinting disturbances and underlying genetic defects in patients with chromosome 11p15.5 related imprinting disorders, Epigenetics, 2018,* 13, pp. 897-909.

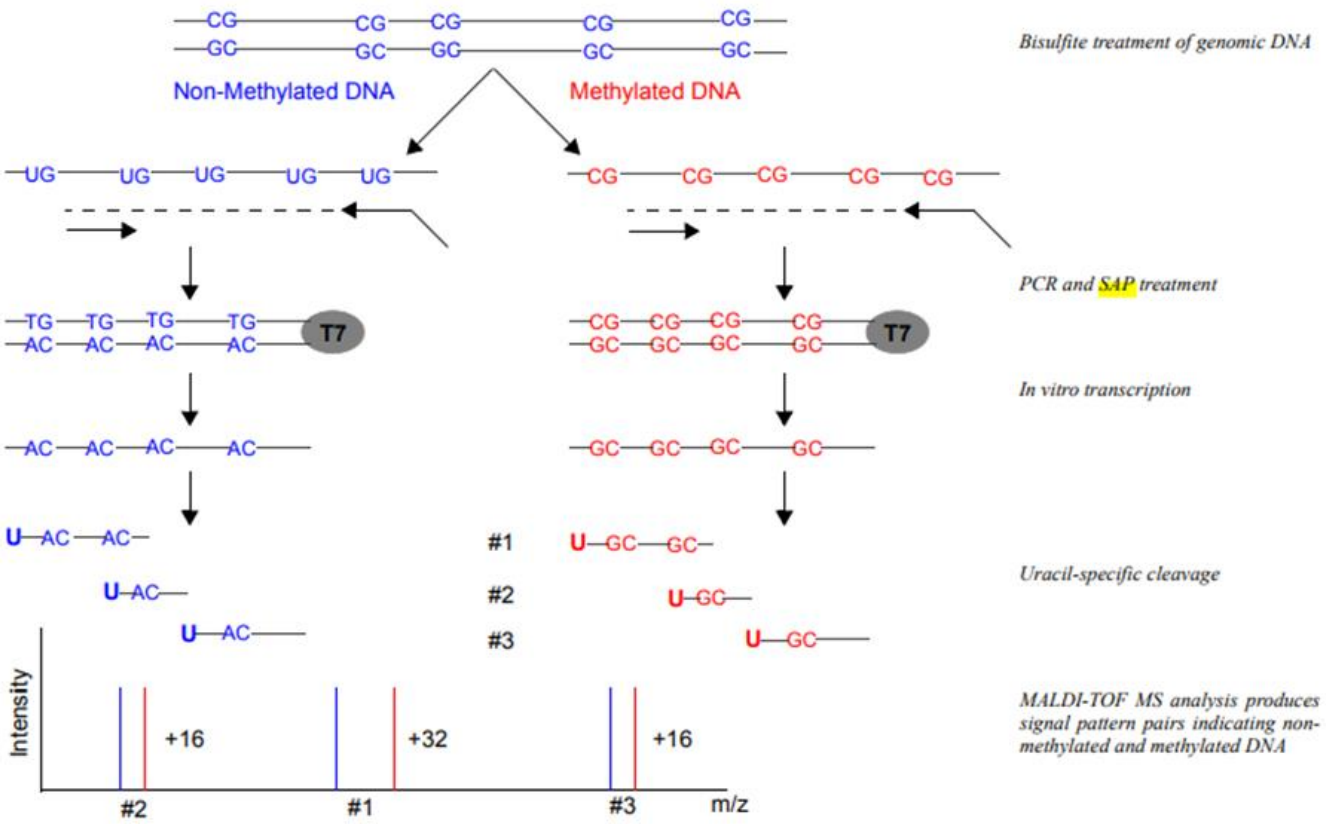
samples were then prepared for quantitative methylation analysis through the MassARRAY System (Agena Bioscience), a highly sensitive technique for measuring DNA methylation levels[183]. Prior to this analysis, the samples were treated with Shrimp Alkaline Phosphatase (SAP) to eliminate unincorporated nucleotides from the PCR process. The DNA samples were then transcribed into RNA and fragmented using restriction enzymes.

These RNA fragments were analyzed using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry), a powerful tool for assessing the molecular mass of DNA fragments. This method enables the accurate determination of the nucleotide sequence of the fragments, as the mass of the fragment corresponds directly to its nucleotide composition. By comparing the ratio of adenine-cytosine (AC) to guanine-cytosine (GC) pairs, the methylation status of each gene can be precisely determined.

The methylation data are presented as a quantitative value ranging between zero and one. A value of zero indicates that the gene is fully unmethylated, while a value of one represents complete methylation of the gene. Intermediate values between 0 and 1 reflect partial methylation, indicating that a proportion of the cell population exhibits methylation at the specific site. This variability is expected, as different levels of methylation can coexist within the same organism and within the same cell population.

[183] M. EHRICH, *et al. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry, Proc Natl Acad Sci U S A, 2005*, 102, pp. 15785-90.

For a more comprehensive discussion of the method, including technical data and validation, readers are referred to the work of Tabano et al.[184].



Overview of the quantitative methylation analysis process

The statistical analysis of the study results was performed both on the data obtained from the questionnaires and on the evaluation of differences in DNA methylation levels between women who experienced sexual violence and the control group. For the questionnaire data, univariate descriptive analyses were conducted to summarize key information, including socio-demographic

[184] S. TABANO, *et al. Analysis of BRCA1 and RAD51C Promoter Methylation in Italian Families at High-Risk of Breast and Ovarian Cancer, Cancers (Basel),* 2020, 12, p. 910.

characteristics and other factors that could influence epigenetic patterns, such as drug therapies, substance use, and pre-existing medical conditions. These analyses were conducted separately for the trauma-exposed and non-trauma-exposed groups to identify any potential distinctions.

The study variables were categorized as either categorical or continuous. Categorical variables, such as demographic factors and medical history, were presented as frequencies and proportions. Continuous variables, such as age or levels of exposure to risk factors, were summarized using means and standard deviations, or medians and interquartile ranges, depending on the distribution of the data. Differences in baseline characteristics between the two groups were assessed using appropriate statistical tests. For data with skewed distributions, non-parametric tests, such as the McNemar test or Wilcoxon rank-sum test, were applied. For normally distributed data, parametric tests such as the chi-square test or Student's t-test were used to evaluate group differences.

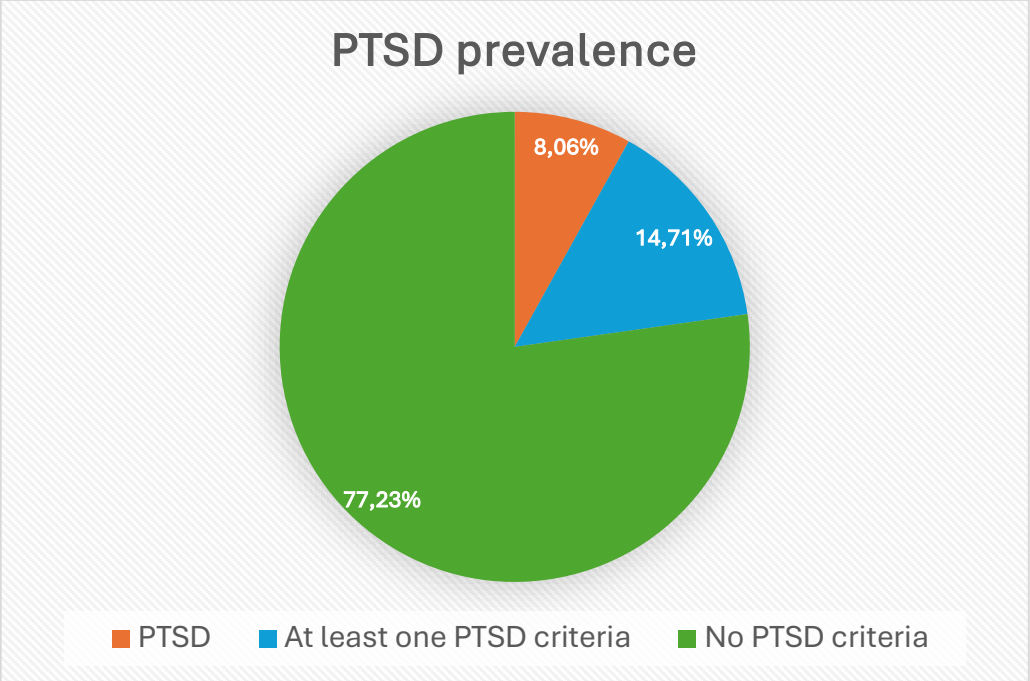
In relation to the methylation analysis, the study focused on comparing methylation levels of the selected genes between the two groups. The analysis involved calculating the mean, median, and standard deviation for methylation levels at each gene site. Given that methylation data typically do not follow a normal distribution, non-parametric tests, specifically the Wilcoxon rank-sum test, were employed to assess the statistical significance of differences in methylation levels between the exposed and control groups.

3.4.

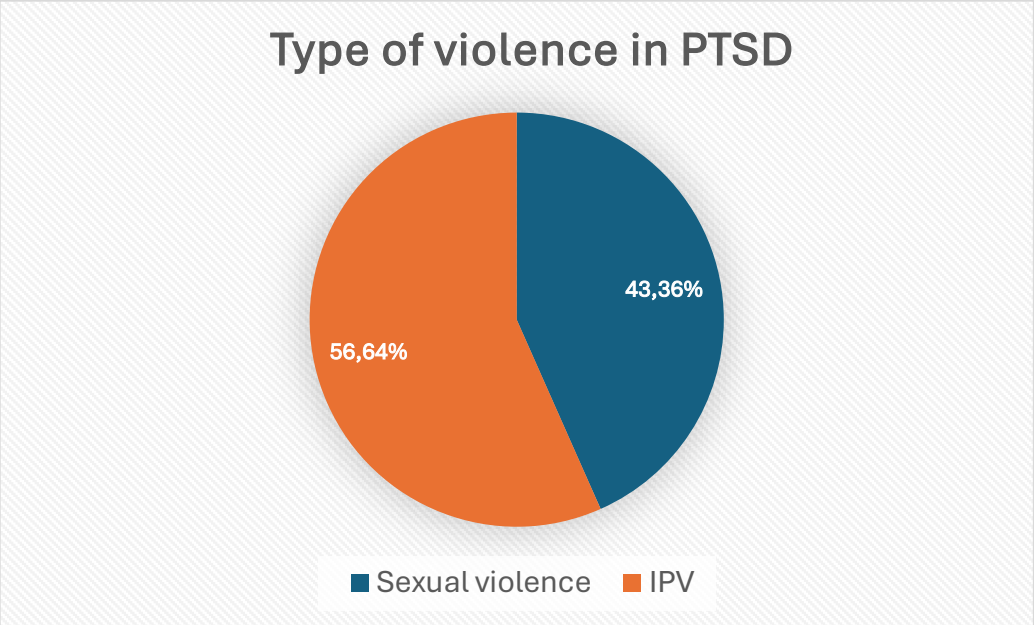
3.5. Results.

In 2019, a total of 914 cases were documented at SVSeD, comprising 420 instances of sexual violence and 494 instances of IPV. Of these, only 496 patients underwent psychological assessments by completing the Post-Traumatic Stress Disorder Checklist-Civilian Version (PCL-C). Among the assessed patients, a diagnosis of PTSD was established in 40 individuals, representing 4.38% of all cases and 8.06% of those who participated in the interview. Specifically, 16 patients (40%) were identified as victims of sexual violence, while 24 patients (60%) were classified as victims of IPV.

Furthermore, 73 patients exhibited one or more diagnostic criteria associated with PTSD during the evaluation, which corresponds to 7.99% of all cases and 14.71% of those who participated in the interview. Within this group, 33 patients (45.2%) were victims of sexual violence, and 40 patients (54.8%) were victims of IPV. When considering both confirmed PTSD diagnoses and patients meeting at least one diagnostic criterion, 43.36% were victims of sexual violence and 56.64% were victims of IPV.

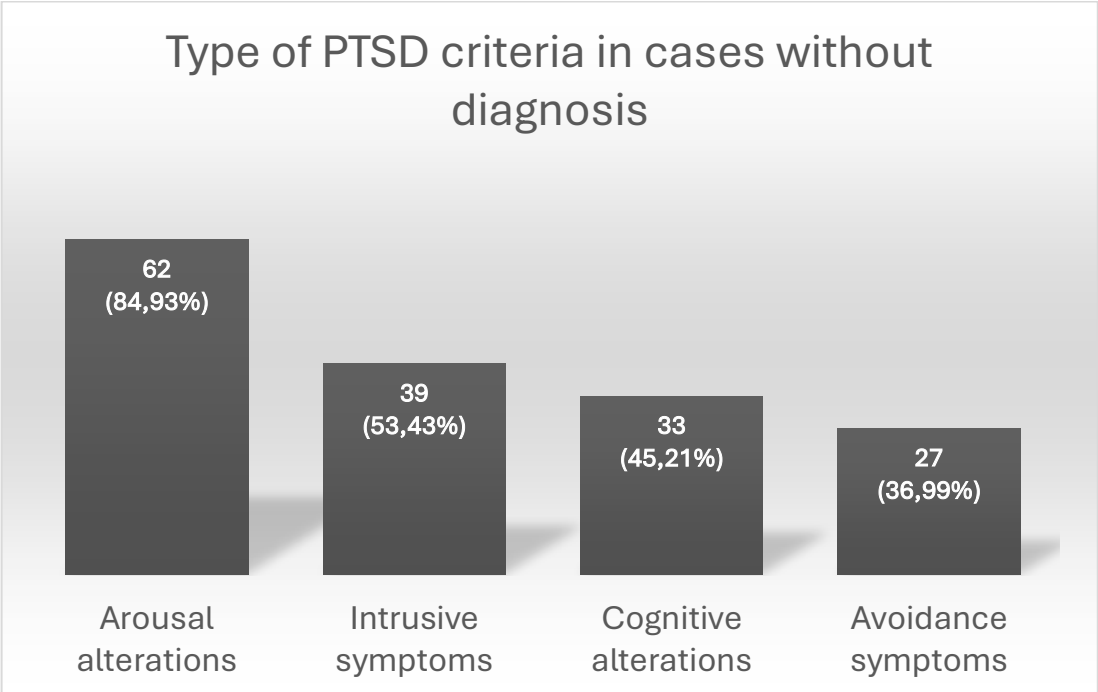


PTSD prevalence calculated on accesses with psychological examination to SVSeD 2019

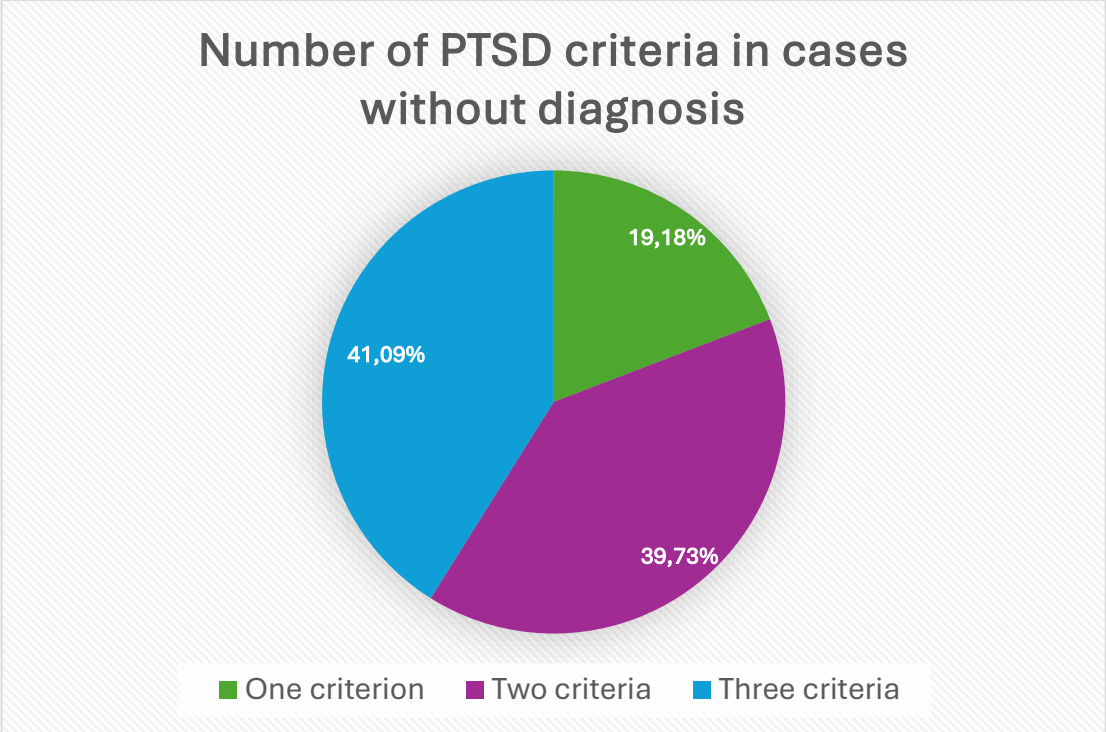


Type of violence experienced in individuals with PTSD or at least one criterion of PTSD in the year 2019 on access to SVSeD

Among the patients who did not meet the diagnostic criteria for PTSD, the most frequently observed symptoms included: 62 instances of arousal alterations (84.93%), 39 cases of intrusive symptoms (53.43%), 33 instances of cognitive alterations (45.21%), and 27 cases of avoidance symptoms (36.99%). Additionally, 14 patients (19.18%) presented with only one criterion, 29 patients (39.73%) met two criteria, and 30 patients (41.09%) exhibited three criteria.



Type of criteria found in subjects with at least one criterion of PTSD in the year 2019 on accesses to SVSeD



Number of criteria found in subjects with at least one criterion of PTSD in the year 2019 on accesses to SVSeD

A total of 62 patients were enrolled in the epigenomic study, with 21 (33.87%) completing the 6-month follow-up, 10 (16.13%) the 12-month follow-up, and 3 (4.84%) the 18-month follow-up. Of the enrolled patients, 49 had experienced IPV and 13 had been exposed to sexual violence. Additionally, 50 participants constituted the comparison group for analysis.

Statistical analysis was conducted solely on samples collected at the patients' initial visit to the anti-violence service (Time Zero, T=0), as the small sample sizes from subsequent follow-ups precluded meaningful statistical evaluation. Due to the limited number of samples, the T=0 samples were not subdivided into groups based on the type of

violence (sexual or IPV). Instead, all cases of violence were analyzed collectively in the initial analysis.

Below are the methylation level values of the genes under investigation, calculated by presenting the distribution (median and interquartile range) for both the violence-exposed group and the comparison group.

CASES (n=62)	I QUARTILE	MEDIAN	III QUARTILE
ADCYAP1	**0.11**	**0.13**	**0.15**
BDNF	**0.07**	**0.08**	**0.10**
CRHR1	**0.07**	**0.08**	**0.08**
DRD2	**0.10**	**0.14**	**0.17**
FKBP5	**0.04**	**0.05**	**0.05**
IGF2	**0.49**	**0.53**	**0.56**
LSD1	**0.04**	**0.04**	**0.05**
NR3C1	**0.03**	**0.04**	**0.05**
PRTFDC1	**0.03**	**0.04**	**0.05**
SLC6A4	**0.04**	**0.05**	**0.05**

CTRLS (n=50)	I QUARTILE	MEDIAN	III QUARTILE
ADCYAP1	**0.11**	**0.13**	**0.16**
BDNF	**0.06**	**0.07**	**0.08**
CRHR1	**0.07**	**0.08**	**0.08**
DRD2	**0.08**	**0.11**	**0.14**
FKBP5	**0.05**	**0.05**	**0.06**
IGF2	**0.45**	**0.48**	**0.52**
LSD1	**0.03**	**0.04**	**0.06**
NR3C1	**0.03**	**0.04**	**0.05**
PRTFDC1	**0.04**	**0.04**	**0.05**
SLC6A4	**0.04**	**0.05**	**0.05**

Median and interquartile range of the values of the methylation levels of the genes considered: at the top the values of the cases of violence (CASES), at the bottom those of the comparison group (CTRLS).

The analysis reveals that methylation levels of the candidate genes in the violence-exposed group, when compared to the comparison group, showed an increase in BDNF, DRD2, and IGF2; remained unchanged for ADCYAP1, LSD1, CRHR1, NR3C1, SLC6A4, and PRTFDC1; and exhibited a slight decrease only in FKBP5.

Additionally, the statistical significance of the differences between groups was assessed using Wilcoxon's non-parametric rank-sum test. To account for multiple comparisons, the Bonferroni correction was applied, setting the threshold for statistical significance at a p-value <0.005 (0.05/10). A p-value below this threshold indicates a statistically

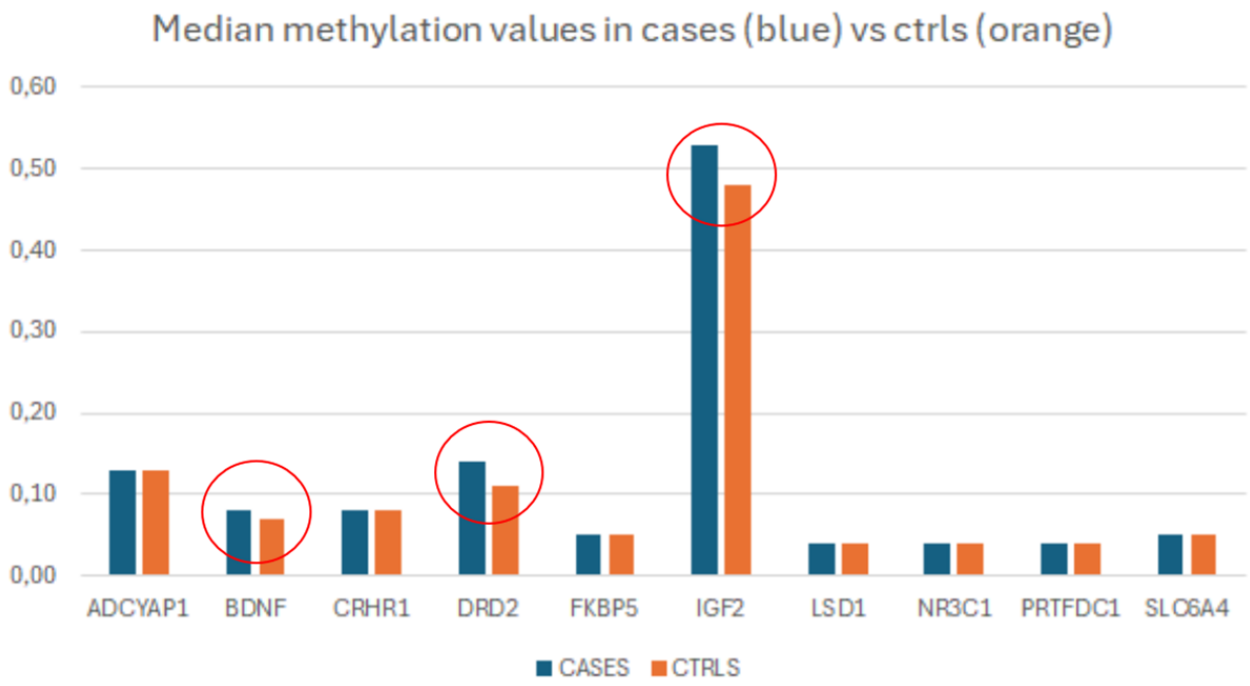
significant difference in the median methylation levels between the groups.

The obtained values are presented below.

P-VALUE pz vs ctrls (Wilcoxon rank-sum)	
ADCYAP1	0.307
BDNF	0.002
CRHR1	0.496
DRD2	0.003
FKBP5	0.018
IGF2	0.001
LSD1	0.186
NR3C1	0.188
PRTFDC1	0.271
SLC6A4	0.347

The p-values for the methylation levels of the analyzed genes between the violence-exposed group and the comparison group are provided below, with statistically significant differences highlighted in yellow.

Significant differences in methylation were observed in the genes BDNF, DRD2, and IGF2, indicating a clear distinction between the two groups. The remaining genes did not demonstrate statistically significant differences in methylation levels.



Values of the average methylation levels of the genes considered between victims of violence and comparison group: the methylation levels of genes with statistically significant differences are highlighted.

3.5 Discussion

Sexual violence represents a critical issue for society. As previously noted, it is estimated that approximately one in three women in Italy will experience sexual violence during their lifetime. In response to this alarming phenomenon, specialized anti-violence centers have been established, particularly in larger cities. A key example is the SVSeD center at the Mangiagalli Clinic in Milan. These centers serve as crucial institutions for observing and addressing violence and are indispensable for any comprehensive study of the issue.

Victim care, as highlighted by the WHO, encompasses both clinical and medico-legal support. A key objective is to enhance the approach to, and quality of care provided to victims. It is important to emphasize that the services offered at Milan's anti-violence centers extend beyond addressing sexual violence; they also focus on instances of ill-treatment

(IPV). Distinguishing between these two forms of violence is essential: sexual violence often involves a singular incident, whereas ill-treatment typically entails repeated abusive actions over time. Both forms of violence can have distinct impacts on women's health, but both can also lead to the development of PTSD.

According to the DSM-5, PTSD is defined as "*an anxiety disorder arising from high-impact emotional experiences and characterized by objective severity*". In cases of rape, approximately 50% of women develop PTSD, with symptoms often emerging within the first two weeks following the traumatic event.

An analysis of PTSD prevalence among patients at the Milan anti-violence center in 2019 revealed that approximately 8% of patients developed overt PTSD, while around 15% exhibited at least one diagnostic criterion for the disorder. When examining the types of violence endured, the data shows that the two categories—IPV (57%) and sexual violence (43%)—are almost equally represented. Unfortunately, PTSD is likely underdiagnosed among victims of violence. This is partly because about half of the patients accessing anti-violence services do not attend psychological interviews, thereby limiting the ability to assess them comprehensively. Moreover, identifying the presence of one or more diagnostic criteria does not preclude the future development of additional PTSD symptoms, particularly for those patients who initially meet two or three criteria.

To improve diagnostic accuracy and provide more effective therapeutic support, continuous psychological care is crucial. Ongoing care can help detect emerging PTSD symptoms, even in individuals

who may not initially present with the full range of diagnostic features. This proactive approach would facilitate better long-term outcomes for victims of violence.

A first aid center, such as the anti-violence center in Milan, cannot be expected to bear the substantial and complex responsibility of long-term therapeutic support, as its primary mission is to provide immediate assistance in the aftermath of a violent event. Given these constraints, it is imperative that an external service be established to manage the broader and ongoing care needs of victims.

While the epigenetics of PTSD have been extensively studied in populations such as war veterans[185] and genocide survivors[186], there remains a significant gap in research focused specifically on individuals who develop PTSD as a result of sexual violence. This gap in the literature served as the impulse for the current thesis project, which integrates the expertise of a multidisciplinary team, including gynecologists, forensic doctors, psychologists, molecular biologists, epidemiologists, and statisticians.

The project is defined by a dual approach, with two interconnected components: first, the collection of comprehensive anamnestic-clinical data and the administration of questionnaires to assess the clinical and social circumstances of the victims; and second, the collection of biological samples. The goal of analyzing these biological samples is to identify epigenetic modifications linked to PTSD, which arises from sexual violence, and to correlate these findings with the victims'

[185] R., YEHUDA, *et al. Lower methylation of glucocorticoid receptor gene promoter 1F in peripheral blood of veterans with posttraumatic stress disorder, Biol Psychiatry*, 2015, 77, pp. 356-364.
[186] V. VUKOJEVIC, cited above, p. 72.

personal histories. Once such epigenetic changes are identified, the aim is to derive potential biological markers for PTSD. Moreover, by conducting follow-up assessments over a period of up to 18 months, the study seeks to determine whether these epigenetic modifications remain stable or shift following clinical-psychological interventions. This will also provide insight into which therapeutic strategies are most effective in supporting victims.

A key methodological decision in this project was to separate the process of obtaining informed consent into two stages. Initially, upon the patient's arrival, consent is obtained solely for the collection and storage of biological samples. At a later stage, during the psychological interview, the study itself is explained in greater detail, and specific consent for participation in the research is collected. This approach has proven highly effective, as it reduces the immediate burden on victims during the acute first aid process, thus fostering higher participation rates. By giving victims time to fully understand the study's aims, this method encourages their involvement, empowering them to contribute to research that may ultimately benefit future victims of violence.

Despite strong initial participation, the follow-up phase of the study proved challenging, with only 35% of participants attending the first follow-up appointment at six months, less than 20% at twelve months, and approximately 5% at the final follow-up at eighteen months. This high dropout rate is likely attributable to the psychological vulnerability of victims of violence, which complicates long-term engagement in research. As a result, a comprehensive epigenomic and statistical prospective analysis could not be conducted. These findings highlight

the need to consider enrolling participants in future prospective studies from facilities that provide sustained care, such as shelters, community support centers, or through general practitioners, rather than relying solely on anti-violence centers focused on immediate intervention.

As noted earlier, this thesis project serves as a pilot study within the broader EpiWE project, and the limited number of biological samples available for epigenetic investigation presented significant constraints. This limitation prevented the division of samples into meaningful subgroups based on the type of violence experienced, reducing the statistical power of the results. It is important to recognize that epigenetic patterns may differ substantially between individuals who have experienced a single, recent incident of sexual assault (i.e., time zero) and those who have endured prolonged exposure to IPV (i.e., time other than zero). With a larger cohort of participants, future studies will be able to differentiate between these groups and incorporate additional variables highlighted in the questionnaires, such as ethnicity, physical injuries, and other factors.

The initial results of the study were based on a comparison between 62 first-time victims of violence and 50 women with no history of major traumatic events. However, the small size of the comparison group underscores the need to increase its numbers to match those of the study cohort. Despite these limitations, the study was able to conduct a meaningful statistical analysis. The comparison of average methylation levels between the group of rape victims and the control group revealed increased methylation levels in three genes, BDNF, DRD2 and IGF2. Conversely, methylation levels for ADCYAP1, LSD1, CRHR1,

NR3C1, SLC6A4, and PRTFDC1 were similar between the two groups, while FKBP5 showed decreased methylation.

Using a Wilcoxon's non-parametric rank-sum test, statistically significant candidate genes were identified, specifically BDNF, DRD2 and IGF2. These genes exhibited significant increases in methylation, which corresponded to a decrease in their gene expression.

The BDNF gene encodes brain-derived neurotrophic factor, a critical regulator of synaptic transmission and neuroplasticity, playing a central role in processes such as stress response, learning, and memory[187]. Experimental studies in murine models have demonstrated that physical exercise induces hypomethylation of the BDNF gene promoter, leading to increased expression of this gene[188]. Such evidence positions BDNF as a potential therapeutic target. Our findings further strengthen the association between hypermethylation of this locus and stress-related conditions, mirroring epigenetic patterns observed in veterans diagnosed with PTSD[189].

Regarding the DRD2 gene, our results are consistent with previous research. Groleau et al. found that women with bulimia spectrum disorders, particularly those with a history of childhood sexual abuse, exhibited increased methylation of the DRD2 gene[190]. This epigenetic modification may downregulate the activity of dopamine D2 receptors,

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[190] P. GROLEAU, *et al. Methylation of the dopamine D2 receptor (DRD2) gene promoter in women with a bulimia-spectrum disorder: Associations with borderline personality disorder and exposure to childhood abuse, J Psychiatr Res*, 2014, 48, pp. 121-127.

suggesting that early-life trauma can result in lasting epigenetic alterations at this locus.

The third locus identified as significantly hypermethylated was IGF2, an imprinted gene encoding insulin-like growth factor 2, known for its essential role in fetal and placental development[191]. Beyond its developmental functions, IGF2 is implicated in cognitive processes such as memory, and in neuropsychiatric disorders like depression and autism. Preclinical studies have shown that IGF2 administration enhances memory performance and reverses cognitive decline, motor impairments, and seizures in animal models[192]. Moreover, Mellott et al. demonstrated that intracerebral injection of IGF2 in mice altered hippocampal BDNF levels, indicating a functional interaction between these two genes[193]. Our findings confirm the significance of these epigenetic changes in affected individuals when compared to controls. The STRING database further supports this interaction, identifying co-expression of BDNF and DRD2 proteins in the hippocampus[194].

Collectively, these findings highlight the critical role of BDNF expression in response to IGF2 signaling and suggest a functional link between BDNF and DRD2. Although preliminary, these results offer valuable insights into potential epigenetic markers in genes involved in

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[191] S. TABANO, *et al. Epigenetic modulation of the IGF2/H19 imprinted domain in human embryonic and extra-embryonic compartments and its possible role in fetal growth restriction, Epigenetics,* 2010, 5, pp. 313-324.

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[194] Global Biodata Coalition and ELIXIR. © STRING CONSORTIUM 2023. STRING. Protein-Protein Interaction Networks. Functional Enrichment Analysis, 2023, https://string-db.org/ (20/10/2024).

brain plasticity, which may influence learning and memory processes under stress, particularly in cases of PTSD arising from sexual violence or intimate partner violence.

The significance and biological implications of these epigenetic modifications remain unclear due to the limited sample size, which prevents definitive conclusions. However, within the preliminary scope of this study, the findings represent an important step in demonstrating that traumatic events, such as violence, can induce epigenetic alterations in affected women. Although certain genes did not reach statistical significance, it would be premature to exclude them from future analyses. With an expanded sample size, these genes may play a critical role in understanding the epigenetic landscape of trauma.

This study can be regarded as a pioneering effort in using epigenetic markers to investigate the biological impact of violence on women. Given the relevance and significance of this research, future studies should aim to expand beyond a candidate gene approach to explore methylation patterns across the entire genome (methylome)[195] and include the analysis of micro-RNAs (miRNAs)[196]. These advancements could lead to the development of valuable biomarkers for diagnosing trauma, evaluating therapeutic interventions, and, once the biological consequences of these epigenetic changes are better understood, informing medico-legal assessments related to the harm suffered by victims or serving as evidence in legal proceedings.

[195] M.W. LOGUE, cited above, p. 77.
[196] C.G. MARTIN, cited above, p. 78.

3.6. Study Limitations.

The relatively small sample size of both victim and control groups in our study has constrained our ability to establish a statistically significant correlation between the clinical profiles of the victims and their respective epigenetic signatures. A more robust sample would be necessary to detect subtle but potentially meaningful associations. Furthermore, the retrospective nature of the PTSD prevalence analysis introduces inherent limitations commonly associated with this type of study design. Retrospective studies often rely on pre-existing medical records, which are prone to incomplete data capture, as they depend heavily on the accuracy and thoroughness of patient examination and documentation. This often results in high rates of missing information, particularly in conditions like PTSD.

Moreover, errors or inconsistencies in the original data collection process, such as misclassification or inaccurate reporting, further compound the challenges in obtaining reliable insights from retrospective analyses. Additionally, retrospective studies lack the prospective control of variables, making it difficult to account for confounding factors that may influence both the clinical presentation of PTSD and the epigenetic alterations under investigation.

In light of these limitations, our findings should be interpreted with caution. While suggestive trends may emerge, further studies with larger, well-characterized cohorts and prospective designs are essential to rigorously evaluate the potential link between epigenetic modifications and PTSD risk among victims of trauma. This would allow for a more comprehensive analysis that accounts for confounding

variables, longitudinal changes, and more accurate clinical correlations. Such improvements in study design would enhance the reliability of conclusions drawn from the data, facilitating a better understanding of the relationship between trauma exposure, epigenetic changes, and PTSD development.

3.7. Conclusions.

The present study identified potential epigenetic modifications associated with the experience of violence, though further investigation is required to substantiate these correlations. To achieve more robust and generalizable findings, it will be essential to increase the sample size and enhance the methodological framework, incorporating prospective evaluations. In parallel, there is a clear need to strengthen psychological and clinical support services for victims, better integrating these services with immediate first-aid interventions. A more structured and comprehensive approach would not only improve the quality of care and safeguard the health of victims, but also foster higher adherence to research protocols.

Building on the preliminary findings of this study, the EpiWE (Epigenetics for WomEn) project will expand into a multi-centre study aimed at enrolling a larger cohort of victims. The primary objective will be to explore correlations between PTSD symptoms in victims of violence and specific epigenetic signatures. This investigation will employ whole-epigenome approaches, examining not only DNA methylation patterns but also other forms of epigenetic regulation, such as histone modifications and microRNA-mediated gene silencing.

Through this comprehensive approach, we aim to identify epigenetic signatures linked to violence that can be translated into clinical practice. These signatures may improve the prediction of the severity of health outcomes resulting from gender-based violence and stress. Furthermore, the identification of such markers may contribute to understanding their role in other biological pathways, linking the long-term effects of violence to various health outcomes, including cancer, cardiovascular disease, and autoimmune disorders.

This knowledge will have significant implications from both clinical and medico-legal perspectives. Clinically, it will enable the refinement and personalization of psychological and therapeutic interventions, making them more targeted and effective. Medico-legally, understanding the harmful consequences of epigenetic alterations could provide a more precise basis for assessing personal injury.

From a legal standpoint, these findings could be groundbreaking. While proving the occurrence of violence in court can be challenging, particularly in cases where the victim's testimony is the primary evidence, the identification of specific epigenetic signatures linked to violence could serve as critical corroborative evidence. Such biomarkers could support the victim's account, enhancing its credibility and facilitating the delivery of justice by holding perpetrators accountable. However, it is important to acknowledge that the path toward legal applicability is long and requires extensive validation of scientific results, backed by a strong foundation in the existing literature.

In the end, while the current findings are promising, significant work remains to fully validate these epigenetic markers and establish their utility in both clinical and legal contexts. With continued research and validation, epigenetic analyses could one day play a crucial role in both improving victim care and strengthening legal outcomes in cases of violence.

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