IN THE UNITED STATES DISTRICT COURT
FOR THE MIDDLE DISTRICT OF TENNESSEE

BONGO PRODUCTIONS, LLC, ROBERT BEINSTEIN, SANCTUARY PERFORMING ARTS, LLC, and KYE SAYERS,

Plaintiffs,

v.

CARTER LAWRENCE, Tennessee State Fire Marshal, in his official capacity, CHRISTOPHER BAINBRIDGE, Director of Codes Enforcement, in his official capacity, GLENN R. FUNK, District Attorney General for the 20th Judicial District, in his official capacity, and NEAL PINKSTON, District Attorney General for the 11th Judicial District, in his official capacity,

Defendants.

CIVIL ACTION
CASE NO. 3:21-cv-00490
JUDGE TRAUGER

RESPONSE IN OPPOSITION TO PLAINTIFFS' MOTION FOR A PRELIMINARY INJUNCTION

Defendants Carter Lawrence, Christopher Bainbridge, Glenn R. Funk, and Neal Pinkston, in their official capacities only, oppose Plaintiffs' Motion for a Preliminary Injunction. (DE 6.) The Motion should be denied because Plaintiffs have not carried their heavy burden to establish an entitlement to the "extraordinary and drastic remedy" of preliminary relief. *Enchant Christmas Light Maze & Mkt. Ltd. v. Glowco, LLC*, 958 F.3d 532, 539 (6th Cir. 2020) (quoting *Mazurek v. Armstrong*, 520 U.S. 968, 972 (1977)).

BACKGROUND

On March 29, 2021, and April 29, 2021, the General Assembly passed House Bill 1182/Senate Bill 1224 by overwhelming majorities in both Houses. Governor Lee signed House Bill 1182 into law on May 17, 2021, as Public Chapter 453 ("the Act"). The Act furthers the

State's interests in informing persons patronizing buildings open to the public of the building operator's bathroom policy should it deviate from any existing bathroom signage designating a bathroom as available only to persons of a specific biological sex. Plaintiffs seek to enjoin the enforcement of the entire Act, which took effect on July 1, 2021.

A. The Contested Statute.

As pertinent here, the Act applies to all businesses and entities within the State that are open to the public and, "as a matter of formal or informal policy, allow[] a member of either biological sex to use any public restroom within the building or facility." (DE 1-1, § 1(a).)

"Public restroom," as defined by the Act, includes restrooms, locker rooms, shower facilities, dressing areas, and any similar facility that is "open to the general public," "designated for a specific biological sex," and constitutes a "facility or area where a person would have a reasonable expectation of privacy." (DE 1-1, §§ 1(d)(2)(A)(i)-(iii).) However, the term "[p]ublic restroom" does not include single-occupancy restrooms or family restrooms "intended for use by either biological sex." (DE 1-1, § 1(d)(2)(B).)

Business and entities that are subject to the Act must post signage that is easily visible to people entering the restroom[1] and that informs the public that "THIS FACILITY MAINTAINS A POLICY OF ALLOWING THE USE OF RESTROOMS BY EITHER BIOLOGICAL SEX, REGARDLESS OF THE DESIGNATION ON THE RESTROOM." (DE 1-1, § 1(b)(3).)

In short, the Act requires that any entity with multi-user, sex-designated public restrooms—and a policy of allowing all users to use either restroom, regardless of their biological sex—to post signage reflecting that policy on or near the restroom entrances. Qualifying businesses and entities

[1] To that end, the Act also specifically describes the dimensions, coloring, and location of the required signage. (Ex. A, §§ 1(b)(1)-(5).)

that do not comply with the Act's requirements must have received notice of their noncompliance at least 30 days before any action is taken against them. (DE 1-1, § 1(c).)

B. Case History

Plaintiffs—"Tennessee businesses and service providers and their owners"—waited more than a month after the Act's passage to file a Complaint on June 25, 2021, challenging the constitutionality of the Act. (*See generally* DE 1; DE 1, PageID# 1.) Plaintiffs allege that they do not wish to post the signage required by the Act because they perceive that the signage required—which mirrors their bathroom-usage policies—is controversial and stigmatizing. (DE 1, PageID# 2.) They allege that by requiring them to post this signage, the Act compels speech in violation of the First Amendment to the United States Constitution. (DE 1, PageID# 18.) Contemporaneously with the filing of their Complaint, Plaintiffs moved for a preliminary injunction "enjoining enforcement of H.B. 1182/S.B. 1224, 112th Gen. Assemb., 1st Reg. Sess. (Tenn. 2021)." (*See generally* DE 6.)

ARGUMENT

The preliminary relief Plaintiffs seek is an "'extraordinary and drastic remedy, one that should not be granted unless the movant, *by a clear showing*, carries the burden of persuasion." *Glowco*, 958 F.3d at 539 (emphasis in original) (quoting *Mazurek*, 520 U.S. at 972). To determine whether such extraordinary relief is warranted, courts consider four factors: "(1) whether the movant has a strong likelihood of success on the merits; (2) whether the movant would suffer irreparable injury absent the injunction; (3) whether the injunction would cause a substantial harm to others; and (4) whether the public interest would be served by the issuance of an injunction." *Am. Civil Liberties Union Fund of Mich. v. Livingston Cnty.*, 796 F.3d 636, 642 (6th Cir. 2015). Because Plaintiffs have not made the requisite clear showing that they are entitled to preliminary

relief, their motion for a temporary restraining order and/or preliminary injunction should be denied.

I. Plaintiffs Are Unlikely to Succeed on the Merits.

A. The Court Lacks Jurisdiction to Issue a Preliminary Injunction

1. The Plaintiffs Lack Standing Because their Alleged Injury-in-Fact is not Imminent.

Article III of the United States Constitution limits the jurisdiction of federal courts to cases and controversies. U.S. Const. art. 3, § 2. The doctrine of standing is "an essential and unchanging part of the case-or-controversy requirement," and defines the boundaries of jurisdiction under Article III by "identifying those disputes which are appropriately resolved through the judicial process." *Lujan v. Defs. of Wildlife*, 504 U.S. 555, 560 (1992). Thus, as a threshold matter, federal courts are "under an independent obligation to examine their own jurisdiction, and standing is perhaps the most important of the jurisdictional doctrines" that a plaintiff must satisfy. *FW/PBS, Inc. v. City of Dallas*, 493 U.S. 215, 231 (1990) (internal quotations and citation omitted); *see Copas v. Lee*, 396 F.Supp.3d 777, 786 (M.D. Tenn. 2019) ("Standing is a 'threshold determinant[ ] of the propriety of judicial intervention.'" (quoting *Warth v. Seldin*, 422 U.S. 490, 518 (1975)).

In determining whether a plaintiff has standing, courts consider whether the plaintiff has alleged "an 'injury in fact' that is 'fairly traceable to the challenged action of the defendant' and is capable of being 'redressed' by the court." *McKay v. Federspiel*, 823 F.3d 862, 867 (6th Cir. 2016) (quoting *Lujan*, 504 U.S. at 560-61). And the plaintiff bears the burden of establishing the presence of all three elements. *Id*. To establish "injury-in-fact," the plaintiff must show he suffered a *concrete* injury that is "actual or imminent, not conjectural or hypothetical." *Lujan*, 504 U.S. at 560. Moreover, the injury alleged must be particularized to the plaintiff—"not a generalized grievance." *Allen v. Wright*, 468 U.S. 737, 751 (1984).

Here, Plaintiffs challenge the Act under the First Amendment, yet they do not allege that the Defendants have actually enforced or will enforce any provisions of the Act against them. "In a pre-enforcement challenge, whether the plaintiff has standing to sue often turns upon whether he can demonstrate an 'injury in fact' before the state has actually commenced an enforcement proceeding against him." *Kiser v. Reitz*, 765 F.3d 601, 607 (6th Cir. 2014). Although Plaintiffs are not required to subject themselves to actual arrest or prosecution as a prerequisite, pre-enforcement challenges typically require "the threatened injury [to be] certainly impending or [that] there is a substantial risk that the harm will occur." *Susan B. Anthony List v. Driehaus*, 573 U.S. 149, 158 (2014) (quoting *Clapper v. Amnesty Int'l USA*, 568 U.S. 398, 414, n. 5 (2013)). Specifically, the Supreme Court has permitted satisfaction of the "injury-in-fact" requirement in the pre-enforcement context when plaintiffs allege "an intention to engage in a course of conduct arguably affected with a constitutional interest, but proscribed by statute, and there exists a credible threat of prosecution thereunder." *Babbitt v. Farm Workers*, 442 U.S. 289, 298 (1979).

Under the Act, "[i]f an entity or business is notified that it is not in compliance with this section, the entity or business has thirty (30) days in which to comply before any action is taken against the entity or business." (DE 1-1, § 1(c).) Plaintiffs in this case "do not want to display the government-mandated warning notice required by the Act." (DE 1, PageID# 2.) They allege that their refusal to comply with the Act could result in imprisonment or fines if they "do not erect the Act's required warning notice after notification that they are not in compliance." *Id*. In other words, Plaintiffs allege that, if they refuse to erect the sign, they *risk* being notified that they are not in compliance with the Act, and, 30 days later, they *could* face penalties. But this abstract risk does not amount to "a credible threat of prosecution" under the Act.

In *McKay*, the plaintiff brought a Section 1983 claim against public officials charged with

enforcing a state court's administrative order prohibiting recording devices in the courtroom. 823 F.3d at 864-65. There, the plaintiff did not allege that he requested or was denied permission to use a recording device, nor that he attempted to enter the courthouse with such a device. Instead, he merely alleged that he did not wish to be subject to contempt, confiscation of a device, a fine, or jail time pursuant to the order. *Id*. at 865-66. In holding that the plaintiff lacked standing, the Sixth Circuit considered a series of factors that, in conjunction with a plaintiff's allegation of a subjective First Amendment chill, could establish injury-in-fact:

> (1) a history of past enforcement against the plaintiffs or others; (2) enforcement warning letters sent to the plaintiffs regarding their specific conduct; and/or (3) an attribute of the challenged statute that makes enforcement easier or more likely, such as a provision allowing any member of the public to initiate an enforcement action…[and] a defendant's refusal to disavow enforcement of the challenged statute against a particular plaintiff.

Id. at 869 (internal citations omitted). Indeed, courts considering credible threats of prosecution in the context of a pre-enforcement First Amendment challenge consistently look to some indication of a threat beyond the simple possibility of enforcement. *Id*; *see also Kiser*, 765 F.3d at 609 (finding a credible threat where the plaintiff received two letters from the defendant warning him that he was in violation of the regulation at issue); *Susan B. Anthony List*, 573 U.S. at 164 (2014) (noting that the plaintiff was previously prosecuted by the defendant for the same sort of speech); *Plunderbund Media, L.L.C v. DeWine*, 753 Fed. App'x. 362, 366-72 (6th Cir. 2018) (finding no credible threat where there was no history of enforcement against defendants or the kind of speech at issue, nothing making the statute easier or more likely to enforce, and no evidence of an intention to enforce).

Here, Plaintiffs do not allege facts that satisfy any of the *McKay* factors, and therefore do not allege a credible threat of prosecution. (*See generally*, DE 1.) Indeed, they do not allege receipt of any warning letters, any attribute of the Act that would make enforcement easier or more

likely,[2] or that Defendants have refused to disavow enforcement against them. Just the opposite is true in Plaintiff Bongo's case: Defendant District Attorney General Funk has made public statements expressing his intention to disavow enforcement of the Act in Davidson County, where Bongo's business is located. Kimberlee Kruesi, *Nashville DA won't enforce new bathroom sign law*, Associated Press (May 24, 2021), https://apnews.com/article/nashville-laws-government-and-politics-50412b91ca33cc45c426a9b5a89b1133 (Ex. A).

Moreover, as Plaintiffs acknowledge, the Act provides a 30-day cure period for businesses before any action can be taken. (DE 1, PageID# 18.) Yet Plaintiffs do not allege receipt of such notice. In other words, despite Plaintiffs' express desire not to comply with the Act, they cannot be subject to any action for at least 30 days, starting from notice of noncompliance. Thus, because Plaintiffs fail to allege a credible threat of prosecution under the Act, they fail to properly allege injury-in-fact and lack standing to bring this claim.

Attempting to circumvent the normal requirements of standing, Plaintiffs allege that enforcement of the Act "will chill Plaintiffs' speech." (DE 1, PageID# 82.) That is still insufficient to demonstrate standing. *See McKay*, 823 F.3d at 868-69 ("mere allegations of a 'subjective chill' on protected speech are insufficient to establish an injury-in-fact for pre-enforcement standing purposes.") (quoting *Berry v. Schmitt*, 688 F.3d 290, 296 (6th Cir. 2012)). But even accepting Plaintiffs' arguments at face value, the Act does not deter speech, it requires it. Thus, no chilling effect is present here and the ordinary requirements of Article III apply.

Lastly, because Plaintiffs do not allege enforcement of the challenged statute by Defendants, there is no injury for the court to redress in this case. In *California v. Texas*, 593 U.S.

[2] The Act's 30-day notice requirement before Defendants could subject Plaintiffs to penalties under the Act make its enforcement *more* difficult and *less* likely.

___, No. 19-840, 2021 WL 2459255 (June 17, 2021), the Supreme Court considered the standing of several individual plaintiffs to challenge the Affordable Care Act's minimum-coverage requirement. The Court held that those plaintiffs lacked standing because "no unlawful Government action "fairly traceable" to [the challenged statute] caused the plaintiffs'… harm." *Id*. at *5. As the Court pointed out, it has consistently required plaintiffs "to assert an injury that is the result of a statute's actual or threatened enforcement, whether today or in the future." *Id*. The Court further noted that issuing an injunction in a case where plaintiffs were not harmed by actual or threatened injury would ultimately amount to "an advisory opinion without the possibility of any judicial relief." *Id*. at *6 (quoting *Los Angeles v. Lyons*, 461 U.S. 95, 129, (1983) (Marshall, J., dissenting)); *see also Carney v. Adams*, 141 S. Ct. 493, 498 (2020) (Article III "require[s] that a case embody a genuine, live dispute between adverse parties, thereby preventing the federal courts from issuing advisory opinions"). Here, Plaintiffs simply cannot show a relationship between the judicial relief requested and the "injury" they allege because they have not suffered an injury. In effect, Plaintiffs are requesting an advisory opinion under the guise of injunctive relief.

2. Plaintiffs' Claim is Not Ripe.

Like the doctrine of standing, the ripeness doctrine is rooted in Article III limitations on federal-court jurisdiction. However, ripeness distinctly aims at ensuring cases or controversies filed in federal court are timely to help the court "avoid[]…premature adjudication." *Abbott Labs. v. Gardner*, 387 U.S. 136, 148-49 (1967). To show that the claim is ripe for review, a plaintiff must allege more than some future acts or events that "may not occur as anticipated, or at all." *Nat'l Rifle Ass'n of Am. v. Magaw*, 132 F.3d 272, 284 (6th Cir. 1997).

Determining whether a claim is ripe for review requires a court to consider both "the fitness

of the issue for judicial decision and the hardship to the parties of withholding court consideration." *Abbott Labs*, 387, U.S. at 149. Specifically, the Sixth Circuit considers three factors in determining whether a claim is ripe: "(1) the likelihood that the harm alleged will ever come to pass; (2) whether the factual record is sufficiently developed to allow for adjudication; and (3) hardship to the parties if judicial review is denied." *Norton v. Ashcroft*, 298 F.3d 547, 554 (6th Cir. 2002); *see also Ammex, Inc. v. Cox*, 351 F.3d 697, 706 (6th Cir. 2003). In the context of a claim that relies on the threat of injury, instead of an actual one, the standing and ripeness doctrines can be difficult to distinguish. *Airline Pros. Ass'n of Int'l Bhd. of Teamsters, Local Union No. 1224, AFL-CIO v. Airborne, Inc.*, 332 F.3d 983, 988 (6th Cir. 2003). For example, "[a] threatened or imminent injury may satisfy standing's injury-in-fact requirement, yet the claim may still be unripe if the issues are not fit for judicial review, perhaps because future events may greatly affect the outcome of the litigation and the cost of waiting is not particularly severe." *Id*.

Here, as discussed above at I.A.2., Plaintiffs fail to allege an injury beyond potential future events that may not occur as they expect or may not occur at all. Plaintiffs do not know that the Act will be enforced against them, and even assuming it were enforced against them, they can predict neither how it would be enforced nor the effect of its enforcement. This case therefore lacks the factual development necessary for this Court to properly adjudicate Plaintiffs' claim.

Furthermore, Plaintiff Sanctuary Performing Arts LLC, admits that its multi-use bathrooms "do[] not have a sex designation at this time." (DE 1, PageID# 4-5.) Under the Act, "public restroom" is defined, in part, as being "[d]esignated for a specific biological sex." (DE 1-1, § 1(d)(2).) Thus, the Act currently does not apply to Sanctuary's bathrooms. And while Sanctuary alleges that it plans to open a café, which will require sex designation pursuant to local building codes, (DE 1, PageID# 5), Sanctuary must do more than rely on future events that may not occur

as anticipated or not occur at all. A general statement of plans to open a business that would presumably be subject to the Act is insufficient to demonstrate ripeness.

Thus, due to a lack of standing and ripeness that deprives this Court of subject matter jurisdiction, Plaintiffs cannot demonstrate a likelihood of success on the merits, and this Court lacks jurisdiction to enter a preliminary injunction.

B. The Act is Constitutional.

Even if the Court finds that Plaintiffs sufficiently demonstrated standing and ripeness, the Court should decline to enter a preliminary injunction as Plaintiffs are unlikely to succeed in their constitutional challenge.

Plaintiffs raise only one claim challenging the constitutionality of the Act: that the Act violates their First Amendment rights by "compelling them, on pain of criminal penalty, to communicate a misleading and controversial government-mandated message that they would not otherwise display." (DE 1, PageID# 18.) Plaintiffs urge this Court to subject the Act to strict scrutiny. Plaintiffs, though, concede that, as businesses open to the public, they are subject to regulation, including regulations that require them to post certain signage. (DE 7, PageID# 51). Plaintiffs also concede that "notices [that] communicate purely factual and non-controversial speech [] do[] not offend the First Amendment." (DE 7, PageID# 51.)

Plaintiffs' concessions confirm that strict scrutiny is inappropriate. It is well settled, of course, that a State may not "constitutionally require an individual to participate in the dissemination of an ideological message by displaying it on his private property in a manner and

for the express purpose that it be observed and read by the public." *Wooley v. Maynard*, 430 U.S. 705, 713 (1977).

But while the State has "'no power to restrict expression because of its message, its ideas, its subject matter, or its content,'" "[u]nder the First Amendment," it may still "regulate certain aspects of speech." *Thomas v. Bright*, 937 F.3d 721, 729 (6th Cir. 2019) (quoting *Police Dep't of City of Chi. v. Mosely*, 408 U.S. 92, 95 (1972)). And when a statute does not regulate or compel expressive or ideological speech, strict scrutiny is not the applicable constitutional test. Indeed, the federal appellate courts have repeatedly declined to apply strict scrutiny to non-expressive, non-ideological disclosure requirements in the face of First Amendment challenges. *See, e.g., Zauderer v. Off. of Disciplinary Couns. of Sup. Ct. of Ohio*, 471 U.S. 626, 651 (1985) (declining to apply strict scrutiny to commercial speech); s*ee also Nat'l Electric Mfrs. Ass'n v. Sorrell*, 272 F.3d 104 (2d Cir. 2001) (upholding a labeling requirement containing purely factual and uncontroversial speech); *Conn. Bar Ass'n v. United States*, 620 F.3d 81 (2d Cir. 2010) (subjecting disclosure requirements to rational basis review); *N. Y. State Rest. Ass'n v. N. Y. C. Bd. of Health*, 556 F.3d 114 (2d Cir. 2009) (applying rational basis review to government mandated caloric disclosure requirements); *Scope Pictures, of Mo, Inc. v. City of Kan. City*, 140 F.3d 1201 (8th Cir. 1998) (upholding a signage requirement regarding venereal disease where the signage conveyed no political or ideological message); *United States v. Sindel*, 53 F.3d 874, 878 (8th Cir. 1995)

("First Amendment protection against compelled speech. . . has only been found in the context of governmental compulsion to disseminate a particular political or ideological message.").

Thus, because the Act does not require expressive or ideological speech, the deferential standard of rational-basis review applies.

1. The Act Does Not Require Expressive or Ideological Speech Implicating Strict Scrutiny.

Straining to categorize the Act's signage as expressive speech to invoke strict scrutiny, Plaintiffs allege that "biological sex" is a controversial term (DE 7, PageID# 54) that "carries an anti-transgender connotation" with which Plaintiffs do not wish to align themselves. (DE 7, PageID# 58.)

Plaintiffs fail to prove this point. The signage required by the Act is neither ideological nor expressive speech. Ideological speech is speech which conveys a "point of view." *Wooley*, 430 U.S. at 715. An accurate statement of fact—such as the bathroom-usage policy chosen by Plaintiffs—does not communicate an ideological or political message. *See, e.g.*, *Dutchess/Putnam Rest. & Tavern Ass'n, Inc. v. Putnam Cnty. Dep't of Health*, 178 F.Supp.2d 396, 406 (S.D.N.Y. 2001) (holding that state-mandated signs informing patrons of the risk of smoking did not constitute ideological speech and therefore did not violate the First Amendment).

Plaintiffs' sole argument to the contrary is that "sex assigned at birth" is an appropriate term, but "biological sex" is "stigmatizing and isolating [to] transgender Tennesseans." (DE 7-3, PageID# 87-88; *see also* DE 7-2, PageID# 79 ("I [Plaintiff Sayers] immediately understood the term 'biological sex' to be stigmatizing to transgender people and believe clients will as well.").) However, Plaintiffs can only speak for themselves, and fail to demonstrate that their unfounded opinions about the term "biological sex" are widely accepted.

Plaintiffs' evidence in support of their argument is similarly lacking; they do not cite

sufficient evidence that the Act's terminology is widely considered harmful. They instead rely on two articles that discuss the issues surrounding gender identification and the use of terms that target biology versus identity. (*See generally* DE 7-5, 7-6.) Defendants disagree that the use of the term "biological sex" necessarily transforms any reference into political, expressive speech. Ample evidence demonstrates that the term "biological sex" is regularly used in a neutral, objective context.

The academic community, for example, has not adopted Plaintiffs' ill-founded belief that the term "biological sex" is inherently loaded political speech. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) contrasts sex differences from gender differences, explaining that:

> *Gender differences* are variations that result from biological sex as well as an individual's self-representation that includes the psychological, behavioral, and social consequences of one's perceived gender. The term *gender differences* is used in DSM-5 because, more commonly, the differences between men and women are a result of both biological sex and individual self-representation. However, some of the differences are based on only biological sex.

Am. Psychiatric Assoc., *The Diagnostic and Statistical Manual of Mental Disorders* 15 (5th ed. 2013) (Ex. B).

Other academic publications also recognize a distinction between biology and identity. In discussing gender dysphoria in adolescence, Kaltiala-Heino, et al. use the term "biological sex" in the same way as the Act—as a scientific characterization separate and apart from gender identity: "The [DSM-5] defines gender dysphoria (GD) as a condition in which a person has marked incongruence between the expressed or experienced gender and the biological sex at birth." Riittakerttu Kaltiala-Heino et al., *Gender dysphoria in adolescence: current perspectives*, ADOLESCENT HEALTH, MEDICINE AND THERAPEUTICS 2018:9, 31, 31 (2018) (Ex. C). And in analyzing the implications of COVID-19 from a gender perspective, Walter and McGregor explain

that "[s]ex- and gender-based medicine (SGBM) incorporates how biological *sex* and the sociocultural aspects of *gender* affect health and illness." L.A. Walter and A.J. McGregor., *Sex- and Gender-specific Observations and Implications for COVID-19*, WEST J EMERG MED. 21(3):507-509 (2020) (emphasis in original) (Ex. D); *see also* E.P. Scully et al., *Considering how biological sex impacts immune responses and COVID-19 outcomes*, NAT REV IMMUNOL 20, 442–447 (2020) ("Human biological sex plays a fundamental role in heterogeneous COVID-19 outcomes.") (Ex. E); S.L. Klein et al., *Biological sex impacts COVID-19 outcomes*, PLOS PATHOG 16:6 (2020) (Ex. F).

Nor does judicial precedent align with Plaintiffs' value-laden perception of the term "biological sex." For example, the D.C., Second, Third, Fourth, Fifth, Seventh, Eighth, Ninth, and Tenth Circuit Courts of Appeal have all used the term "biological sex." *See, e.g.*, *Doe 2 v. Shanahan*, 917 F.3d 694, 698 (D.C. Cir. 2019); *Able v. United States*, 88 F.3d 1280, 1286 (2d Cir. 1996); *Grimm v. Gloucester Cnty. Sch. Bd.*, 972 F.3d 586, 614 (4th Cir. 2020); *Parents for Priv. v. Barr*, 949 F.3d 1210, 1217 (9th Cir. 2020); *Doe by and through Doe v. Boyertown Area Sch. Dist.*, 897 F.3d 518, 529 (3d Cir. 2018); *Hively v. Ivy Tech Cmty. Coll. of Ind.*, 853 F.3d 339, 347 (7th Cir. 2017); *Cruzan v. Special Sch. Dist, No. 1*, 294 F.3d 981, 983 (8th Cir. 2002); *Jackson v. Valdez*, --- Fed.App'x ---, 2021 WL 1990788, at *5 (5th Cir. 2021); *Etsitty v. Utah Transit Auth.*, 502 F.3d 1215, 1225 (10th Cir. 2007).

The Sixth Circuit has used the term in its opinions as well. *See, e.g.*, *E.E.O.C. v. R.G. & G.R. Harris Funeral Homes, Inc.*, 884 F.3d 560, 578 (6th Cir. 2018) (affirmed by *Bostock v. Clayton Cnty., Ga.*, 140 S.Ct. 1731 (2020)). So too have Tennessee's federal courts. *See, e.g.*, *Doe v. Hamilton Cnty. Bd. of Educ.*, 329 F.Supp.3d 543, 580 (E.D. Tenn. 2018). Indeed, the Supreme Court itself has used the term "biological sex" in its recent opinions. *See, e.g.*, *Farmer*

v. Brennan, 511 U.S. 825, 829 (1994); *Bostock*, 140 S.Ct. at 1752.

If Plaintiffs are correct, then these courts and academics have chosen a "controversial political term, one that has no value or meaning in medicine or science" and one that carries "an anti-transgender connotation." (DE 7-3, PageID 87; DE 7, PageID 58.) Yet, to the contrary, the term "biological sex" in each of these scientific articles and judicial opinions was used objectively and neutrally as a descriptive term to aid the reader's understanding, just as the term is used in the Act. Plaintiffs cannot twist the Act into a requirement that they engage in ideological speech by adopting an idiosyncratic understanding of a term with another widely accepted and commonly understood meaning. Indeed, on Plaintiffs' view, businesses could not even be required to post sex-designations outside their restrooms, because use of the terms "male" and "female" allegedly "ignores entirely the existence of intersex people, by suggesting that there are only two possible sexes." (DE 1, PageID 13.)

As a second try, Plaintiffs assert that they are forced by the Act "to display a government-mandated warning notice with which they disagree." (DE 1, PageID# 2.). But by the Act's plain language, Plaintiffs need only post the required signage if they *agree* with the language set forth by the Act—that "this facility maintains a policy of allowing the use of restrooms by either biological sex, regardless of the designation on the restroom." (DE 1-1, § 1(a) & (b)(3) (capitalization omitted)). Plaintiffs also complain that unless they "guard[] their restroom doors to ask for their birth certificates, inspect anyone's genitals, or interrogate any other aspect of [their patrons'] sex," they would be required to display the signage required by the Act. (DE 1, PageID# 11.). Plaintiffs' characterization of the Act is wrong again. The Act does not require signage where, due to a patron's disregard of sex-designating signs outside a bathroom, the business's implementation of their chosen bathroom-usage policy is imperfect. Instead, the Act merely

applies where the business's bathroom-usage policy mirrors the language of Section (b)(3). (*See* DE 1-1, § 1(a) & (b)(3).)

This distinction is critical. Because Plaintiffs need only comply with the Act if the signage language matches their bathroom-usage policy, the Act can only compel speech that is necessarily accurate. Accordingly, as the Act only requires the disclosure of accurate, non-ideological, and non-expressive speech, strict scrutiny does not apply and the statute need only satisfy the requirements of rational-basis review.

2. The Act Satisfies Rational-Basis Review.

Here, it is evident that the informed-consent provision easily satisfies rational-basis review. Under rational-basis review, a law is presumed constitutional, and "[t]he burden is on the one attacking the legislative arrangement to negate every conceivable basis which might support it." *Heller v. Doe*, 509 U.S. 312, 320 (1993) (internal quotations omitted); *see also Walker v. Bain*, 257 F.3d 660, 668 (6th Cir. 2001) (stating that a statute is subject to a "strong presumption of validity" under rational-basis review and will be upheld "if there is any reasonably conceivable state of facts that could provide a rational basis").

A court conducting a rational-basis review does not sit "as a super legislature to judge the wisdom or desirability of legislative policy determinations" but asks only whether there is some conceivable rational basis for the challenged statute. *Heller*, 509 U.S. at 319. This means that under rational-basis review, it is "'constitutionally irrelevant [what] reasoning in fact underlays the legislative decision.'" *R.R. Ret. Bd. v. Fritz*, 449 U.S. 166, 179 (1980) (quoting *Flemming v. Nestor*, 363 U.S. 603, 612 (1960)).

In enacting the informed-consent provision, the General Assembly and the citizens of Tennessee had "absolutely no obligation to select the scheme" that a court might later conclude

was best. *Nat'l R.R. Passenger Corp. v. A.T. & S.F.R. Co.*, 470 U.S. 451, 477 (1985); *see McGowan v. Maryland*, 366 U.S. 420, 425-426 (1961) ("State legislatures are presumed to have acted within their constitutional power despite the fact that in practice, their laws result in some inequality."). And Tennessee "has no obligation to produce evidence to sustain the rationality of its action; its choice is presumptively valid and 'may be based on rational speculation unsupported by evidence or empirical data.'" *TriHealth, Inc. v. Bd. of Comm'rs*, 430 F.3d 783, 790 (6th Cir. 2005) (quoting *FCC v. Beach Commc'ns, Inc.*, 508 U.S. 307, 315 (1993)).

Here, the rational basis for the Act is readily apparent. Tennessee certainly has a compelling interest in ensuring that patrons are informed of the bathroom-use policy at businesses they frequent—especially when the bathroom-usage policy differs in practice from the existing bathroom signage used by business owners. Many Tennesseans would agree that being "forced to share changing, shower, and bathroom space with members of the opposite sex" does not provide the same "level of privacy and comfort that" a patron could "expect" in facilities separated based on biological sex. *Stuart v. Metro. Gov't of Nashville & Davidson Cnty.*, 679 F. Supp. 2d 851, 854, 859 (M.D. Tenn. 2009) (Trauger, J.), *vacated after settlement*.

And, although not required by rational-basis review, the statute is also narrowly tailored. The Act simply ensures that Tennesseans are informed of a company's policy before they enter a locker room or bathroom. It does not require Plaintiffs to adopt any specific bathroom-usage policy, nor does it blanketly prohibit patrons from using the bathroom contrary to their biological sex. And the Act does not prohibit Plaintiffs from posting additional signs expressing their particular political or social views. Put another way, the law *does* allow Plaintiffs to permit any individual to use whatever restroom they choose, and it *does not* require using only the restroom that corresponds to biological sex. All that is required is that Plaintiffs inform their patrons of their

bathroom-usage policy in the event that Plaintiffs use bathroom signage inconsistent with their chosen usage policy.

As the Act does not require—or inhibit—expressive, untruthful, or ideological speech, it easily satisfies the applicable constitutional standard. Plaintiffs cannot therefore demonstrate a likelihood of success on the merits, and the Court should decline to grant a preliminary injunction.

II. Plaintiffs are Unlikely to Suffer Irreparable Injury Absent an Injunction.

A preliminary injunction is likewise inappropriate as Plaintiffs have failed to make the requisite showing of irreparable harm.

A plaintiff seeking preliminary injunctive relief must demonstrate that irreparable harm is likely in the absence of the requested injunction. *Lyons*, 461 U.S. at 103; *O'Shea v. Littleton*, 414 U.S. 488, 502 (1974). The showing of likely irreparable harm is the single most important prerequisite for issuance of a preliminary injunction. *See* 11A C. Wright, A. Miller, & M. Kane, Fed. Practice and Procedure § 2948.1 (3d ed.). Speculative injury, then, is not sufficient. *Id.* And a preliminary injunction is not warranted to prevent the possibility of some remote future injury—a presently existing actual threat must be shown. *Id.* "Issuing a preliminary injunction based only on a possibility of irreparable harm is inconsistent with our characterization of injunctive relief as an extraordinary remedy that may only be awarded upon a clear showing that the plaintiff is entitled to such relief." *Mazurek*, 520 U.S. at 972.

A. Issuance of a Notice Does Not Constitute Harm.

At this time, Plaintiffs face only the possibility of a notice of non-compliance with the law, but that alone does not constitute harm. Plaintiffs allege that, without immediate relief, they will be required to post a bathroom sign "within thirty days of a citation, or risk the threat of six months' imprisonment and/or a civil penalty of up to $500 . . . ." (DE 7, PageID# 69.) The necessary

condition that must occur before Plaintiffs suffer any injury is that Plaintiffs be "notified that [they] are not in compliance with [the Act]," in which case, they have "thirty (30) days to comply before any action can even possibly be taken against the entity or business." (DE 1-1, PageID# 21.)

Here, there is no irreparable harm if Plaintiffs do as they wish and choose not to post the signage identified by the Act—the only possible action the government could take is issuance of a notice. The receipt of a notice does not constitute harm. Indeed, it does not even rise to the level of "serious or substantial" harm, much less the higher standard of "irreparable" harm that is necessary for injunctive relief. *See, e.g.*, *A. O. Smith Corp. v. F. T. C.*, 530 F.2d 515, 525 (3d Cir. 1976) ("[T]he requisite is that the feared injury or harm be irreparable—not merely serious or substantial."); *Becton v. Thomas*, 48 F. Supp. 2d 747, 762 (W.D. Tenn. 1999) ("A plaintiff's harm is not considered irreparable if "it is fully compensable by money damages.") Plaintiffs have not identified any irreparable injury they will suffer from the mere receipt of a notice.

The Supreme Court's description of the injury-in-fact necessary for standing purposes is also instructive in determining whether Plaintiffs actually face harm at this pre-enforcement juncture: "[a]n allegation of future injury may suffice if the threatened injury is 'certainly impending' or there is a 'substantial risk' the harm will occur." *Driehaus*, 573 U.S. at 158 (quoting *Clapper*, 568 U.S. at 409, 414, n. 5). Plaintiffs have not demonstrated that any notice has issued, much less that prosecution of a violation following issuance of a notice is imminent. In fact, Defendant Glenn Funk, the Nashville District Attorney General, has publicly taken the position that *he categorically will not enforce the law*, and Tennessee District Attorneys General Conference President Amy Weirich stated that the law "doesn't speak to anything having to do with enforcement" and "[t]he way [the law] is written, I don't see anything that *allows or provides the responsibility or right* to go to civil court and ask a judge to enforce it." Kimberlee Kruesi,

Nashville DA won't enforce new bathroom sign law, Associated Press, (May 24, 2021) https://apnews.com/article/nashville-laws-government-and-politics-50412b91ca33cc45c426a9b5a89b1133. (Ex. A)

Illuminating the lack of an imminent, irreparable injury is the delay between the Act's passage into law and the filing of Plaintiffs' complaint. Knowing that the law would become effective on July 1, 2021, Plaintiffs waited nearly a month before filing this lawsuit—only seeking judicial intervention a mere six days before it took effect. . And what could explain their dilatoriness? The 30-day cure period that insulates them from any conceivable harm. In light of the evidence that Plaintiffs do not face actual harm—only the possibility of a notice—and the fact that actual enforcement of the law's penalties is not only attenuated but also apparently uncertain, Plaintiffs' allegation of irreparable harm is at best speculative at this point. "Issuing a preliminary injunction" here "based only on a possibility of irreparable harm is inconsistent with [the Court's] characterization of injunctive relief as an extraordinary remedy that may only be awarded upon a clear showing that the plaintiff is entitled to such relief." *Mazurek*, 520 U.S. at 972.

Finally, the inquiry into irreparable harm necessarily asks whether the Plaintiffs have an adequate legal remedy absent injunctive relief. *Russell v. Ohio, Dep't of Admin. Servs.*, 302 F. App'x 386, 394 (6th Cir. 2008) ("The basis of injunctive relief in the federal courts has always been irreparable harm and inadequacy of legal remedies."). Plaintiffs do have adequate legal remedies: if Plaintiffs do receive a notice and face some actual threat of enforcement, they can seek preliminary-injunctive relief from this Court at a later date during the pendency of this litigation. Accordingly, Plaintiffs have failed to demonstrate the immediate, irreparable harm necessary for a preliminary injunction.

B. The Term "Biological Sex" Does Not Constitute Harm.

Plaintiffs also imply that posting the notice will cause them some form of "reputational

harm" or stigma: "Sanctuary believes that it will lose the customers and supporters it has worked hard to bring to Sanctuary if it displays the warning notice." (DE 7, PageID# 61; *see also* DE 7-1, PageID# 75 ("I believe that posing the warning notice required by [the Act] will offend [Bongo Productions'] staff, customers, friends, and family.").

Again, if Plaintiffs do not post the signage, the only risk at this point is a notice, which as explained above, does not constitute irreparable injury. And upon receipt of a notice, Plaintiffs would still possess meaningful access to the Court to seek preliminary injunctive relief. But *accepting arguendo* that Plaintiffs do post the sign and fear harm, the harm they identify is that the law "requires Plaintiffs to use a phrase that lacks scientific support, and perpetuates the viewpoint that transgender people are not the sex they know themselves to be." (DE 7, PageID# 66.) As described *supra* in I.B.1, the term "biological sex" is hardly the value-laden scarlet letter Plaintiffs imagine. And nothing in the Act prohibits disassociation or disagreement. If Plaintiffs' unfounded fears about using the term "biological sex" truly disturb them, they are free to publicly attribute the required signage to the State and openly disagree with it—perhaps even with a second sign presenting their protected expressive, ideological speech. But even so, an exaggerated parade of horribles about how third parties may perceive their compliance with the Act is a speculative and insufficient showing of harm.

Apart from the terminology of the law, Plaintiffs also suggest that limiting transgender access to the bathroom of choice is harmful. According to Dr. Taylor, "[a] transgender patron should not have to effectively disclose their transgender status by using the designated restroom that matches their sex assigned at birth," and "[a] transgender person should be able to use the restroom that matches their gender identity." (DE 7-3, PageID# 87.) But Dr. Taylor fundamentally misapprehends the Act, rendering her declaration irrelevant here. The Act *does not* require a

patron to use only the restroom that corresponds to biological sex. (*See* DE 1-1.) Plaintiffs, as operators of their businesses, get to decide their own bathroom-usage policy. The Act only requires that Plaintiffs provide notice to patrons when their bathroom-usage policy differs from their existing signage. Nothing in the Act requires that Plaintiffs inquire into the identity of its patrons or force them to use a specific restroom.

Plaintiffs cannot show that the terminology of the Act causes harm. Plaintiffs also cannot demonstrate the Act requires them to limit an individual's access to the bathroom of that individual's choice. Plaintiffs therefore have not demonstrated a concrete and irreparable injury sufficient to justify issuance of an injunction.

III. Issuance of an Injunction Would Harm the State and the Public Interest.

An injunction that prevents a State from upholding a duly enacted law necessarily irreparably harms the State. *See, e.g.*, *Abbott v. Perez*, 138 S. Ct. 2305, 2324 (2018); *Maryland v. King*, 567 U.S. 1301, 1303 (2012) (Roberts, C.J., in chambers) (citation omitted); *Detroit Newspaper Publisher Ass'n v. Detroit Typographical Union*, 471 F.2d 872, 876 (6th Cir. 1972), *cert. denied*, 411 U.S. 967 (1973); *MLZ, Inc. v. Fourco Glass Co.*, 470 F. Supp. 273, 278 (M.D. Tenn. 1978). And where the party opposing equitable relief is the government, consideration of the public interest "merge[s]" with irreparable harm to the government. *Nken v. Holder*, 556 U.S. 418, 435 (2009). The public has a strong interest in laws duly passed by its representative branch of government, and thus the public interest and harm to the State militate against injunctive relief. *Planned Parenthood of Greater Tex. Surgical Health Servs. v. Abbott*, 734 F.3d 406, 419 (5th Cir. 2013). Due to Plaintiffs' delay in seeking a preliminary injunction, the Act is now in effect, so their argument that the equities weigh more in their favor when "a law . . . is not currently in effect," now points in the Defendants' favor. (DE 6, PageID# 41.)

In practice, the Act is designed as a statute of public notice. It performs a limited purpose: informing the patrons of the bathroom-usage policy at Plaintiffs' business locations. It does not require Plaintiffs to adopt any specific bathroom-usage policy, but it does allow patrons to understand Plaintiffs' bathroom-usage policy to the extent it differs from the expectations conferred by Plaintiffs' existing sex-specific bathroom signage. Just as Tennesseans have a strong interest in the effectiveness of their democratically-passed laws, they also have an interest in being accurately informed of the rules adopted by the businesses they patronize.

CONCLUSION

For the foregoing reasons, Defendants respectfully request that this Court deny Plaintiffs' motion for a preliminary injunction.

Respectfully submitted,

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CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of the foregoing Response has been served on the following counsel of record through the Electronic Filing System on this 7th day of July, 2021:

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Nashville DA won’t enforce new bathroom sign law

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May 24, 2021

By KIMBERLEE KRUESIMay 24, 2021

Amy Allen, the mother of an 8th grade transgender son, speaks after a Human Rights Campaign round table discussion on anti-transgender laws Friday, May 21, 2021, in Nashville, Tenn. Conservative lawmakers nationwide introduced a flurry of anti-LGBTQ bills this year, but no state's political leaders have gone further than Tennessee in enacting new laws targeting transgender people. (AP Photo/Mark Humphrey)

NASHVILLE, Tenn. (AP) — Nashville’s top prosecutor said Monday that he will not enforce a newly enacted law that requires businesses and government facilities open to the public to post a sign if they let transgender people use multiperson bathrooms and other facilities associated with their gender identity.

“I believe every person is welcome and valued in Nashville,” Nashville District Attorney General Glenn Funk said in a statement. “Enforcement of transphobic or homophobic laws is contrary to those values. My office will not promote hate.”

Funk's office clarified that this refusal to enforce "transphobic or homophobic laws" specifically included the first-of-its kind measure signed by Republican Gov. Bill Lee earlier this month.

The move, along with the flurry of other anti-transgender laws approved by Lee, has sparked alarm among LGBTQ advocates. Many have decried the latest measure as discriminatory and said the required signs are "offensive and humiliating." The law will go into effect July 1.

However, questions have remained about how specifically it will be enforced.

Republican Rep. Tim Rudd, who sponsored the legislation, told a legislative committee in March that the bill "does not provide any fines or penalties at this point," and the amended version passed by that committee became law. Rudd has also said that the law could be enforced by people filing lawsuits or district attorneys asking a judge to force businesses to comply.

Yet Tennessee District Attorneys General Conference President Amy Weirich argued that the language in the new law "doesn't speak to anything having to do with enforcement."

"The way it's written, I don't see anything that allows or provides me the responsibility or right to go to civil court and ask a judge to enforce it," said Weirich, Shelby County's district attorney.

Lee did not have a strong reaction when pressed by reporters Monday on Funk's refusal to enforce the bathroom sign law.

"I think his decision will be his own," he said. "I signed the law; it's his decision how he wants to respond to it."

Lee's response was markedly different than when Funk announced in September he would not enforce a new law that required abortion providers to tell their patients it may be possible to reverse the action of abortion medication half-way through the procedure. Funk said at the time he believed the law was unconstitutional.

Without naming Funk, Lee's office tweeted that, "A district attorney purposefully disregarding current, duly enacted laws by the legislature is a grave matter that threatens our justice system and has serious consequences."

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Associated Press writer Jonathan Mattise contributed to this report.

DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS

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These three concepts (for which discussion and examples are provided in Section III and the Appendix) suggest cultural ways of understanding and describing illness experiences that can be elicited in the clinical encounter. They influence symptomatology, help seeking, clinical presentations, expectations of treatment, illness adaptation, and treatment response. The same cultural term often serves more than one of these functions.

Gender Differences

Sex and gender differences as they relate to the causes and expression of medical conditions are established for a number of diseases, including selected mental disorders. Revisions to DSM-5 included review of potential differences between men and women in the expression of mental illness. In terms of nomenclature, *sex differences* are variations attributable to an individual's reproductive organs and XX or XY chromosomal complement. *Gender differences* are variations that result from biological sex as well as an individual's self-representation that includes the psychological, behavioral, and social consequences of one's perceived gender. The term *gender differences* is used in DSM-5 because, more commonly, the differences between men and women are a result of both biological sex and individual self-representation. However, some of the differences are based on only biological sex.

Gender can influence illness in a variety of ways. First, it may exclusively determine whether an individual is at risk for a disorder (e.g., as in premenstrual dysphoric disorder). Second, gender may moderate the overall risk for development of a disorder as shown by marked gender differences in the prevalence and incidence rates for selected mental disorders. Third, gender may influence the likelihood that particular symptoms of a disorder are experienced by an individual. Attention-deficit/hyperactivity disorder is an example of a disorder with differences in presentation that are most commonly experienced by boys or girls. Gender likely has other effects on the experience of a disorder that are indirectly relevant to psychiatric diagnosis. It may be that certain symptoms are more readily endorsed by men or women, and that this contributes to differences in service provision (e.g., women may be more likely to recognize a depressive, bipolar, or anxiety disorder and endorse a more comprehensive list of symptoms than men).

Reproductive life cycle events, including estrogen variations, also contribute to gender differences in risk and expression of illness. Thus, a specifier for postpartum onset of mania or major depressive episode denotes a time frame wherein women may be at increased risk for the onset of an illness episode. In the case of sleep and energy, alterations are often normative postpartum and thus may have lower diagnostic reliability in postpartum women.

The manual is configured to include information on gender at multiple levels. If there are gender-specific symptoms, they have been added to the diagnostic criteria. A gender-related specifier, such as perinatal onset of a mood episode, provides additional information on gender and diagnosis. Finally, other issues that are pertinent to diagnosis and gender considerations can be found in the section "Gender-Related Diagnostic Issues."

Use of Other Specified and Unspecified Disorders

To enhance diagnostic specificity, DSM-5 replaces the previous NOS designation with two options for clinical use: *other specified disorder* and *unspecified disorder*. The other specified disorder category is provided to allow the clinician to communicate the specific reason that the presentation does not meet the criteria for any specific category within a diagnostic class. This is done by recording the name of the category, followed by the specific reason. For example, for an individual with clinically significant depressive symptoms lasting 4 weeks but whose symptomatology falls short of the diagnostic threshold for a major depressive episode, the clinician would record "other specified depressive disorder, depressive episode with insufficient symptoms." If the clinician chooses not to specify the

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REVIEW

Gender dysphoria in adolescence: current perspectives

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Abstract: Increasing numbers of adolescents are seeking treatment at gender identity services in Western countries. An increasingly accepted treatment model that includes puberty suppression with gonadotropin-releasing hormone analogs starting during the early stages of puberty, cross-sex hormonal treatment starting at ~16 years of age and possibly surgical treatments in legal adulthood, is often indicated for adolescents with childhood gender dysphoria (GD) that intensifies during puberty. However, virtually nothing is known regarding adolescent-onset GD, its progression and factors that influence the completion of the developmental tasks of adolescence among young people with GD and/or transgender identity. Consolidation of identity development is a central developmental goal of adolescence, but we still do not know enough about how gender identity and gender variance actually evolve. Treatment-seeking adolescents with GD present with considerable psychiatric comorbidity. There is little research on how GD and/or transgender identity are associated with completion of developmental tasks of adolescence.
Keywords: gender dysphoria, gender identity, adolescence, developmental tasks

Gender dysphoria and related concepts

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[1] defines gender dysphoria (GD) as a condition in which a person has marked incongruence between the expressed or experienced gender and the biological sex at birth. This causes clinically significant distress or impairment in social, occupational or other important areas of functioning. Individuals with GD experience a strong desire to be treated as the other gender (or some alternative gender different from their assigned gender) and/or to be rid of their sex characteristics, and/or the strong conviction of having feelings and reactions typical of the other gender (or some alternative gender). The previous diagnostic term, gender identity disorder, was rejected in the DSM-5 to avoid pathologizing identity.

According to the International Classification of Diseases (ICD)-10,[2] transsexualism is defined as a desire to live and be accepted as a member of the opposite sex, usually accompanied by a sense of discomfort with or the inappropriateness of one's anatomical sex and a wish to undergo surgery and hormonal treatment to make the body as congruent as possible with the individual's preferred sex. The forthcoming ICD-11 will reconceptualize gender identity-related diagnoses using gender incongruence as the main term.[3]

In addition to the DSM-5 diagnostic term, gender dysphoria can also refer to anxiety and distress about gender features at large. Gender nonconformity refers to

behaviors and an appearance that are considered atypical of an individual's assigned gender. Gender variance refers to a spectrum of gender experience, in contrast to the dichotomized conception of gender. The term "transgender" is used as an umbrella term to refer to wider variation of gender identities. Not all who identify as transgender or display gender nonconformity or gender variance suffer from dysphoria.

In this article, we use the DSM-5 and ICD-10 terms "gender dysphoria (GD)" and "transsexualism/transsexual", respectively, when referring to diagnosed clinical samples, and also when referring to the literature published when earlier versions of the DSM classification were in use. We use "transgender" to refer to self-identified population samples and "gender dysphoria" to refer to those who present clinical symptoms.

How common are GD and transgender identity among adults and adolescents?

The number of people who seek treatment suggest that male-to-female transsexualism has a prevalence of 6.8/100,000 and female-to-male transsexualism has a prevalence of 2.6/100,000 among adults.[4,5] In the Netherlands, 0.6% of men and 0.2% of women (aged 15–70 years) reported incongruent gender identity and a desire to undergo sex reassignment (SR).[6] Population surveys have suggested that about 0.5% of adults in the general population identify as transgender.[5,7]

The number of adolescents contacting specialized gender identity services has risen considerably over the past decade across Europe and North America.[8,9] No conclusions regarding the prevalence of GD in general or of GD/transsexualism specifically can be drawn based on these increases. Studies using short (one to three item) self-reports of gender identity and its variance suggest that 0.17%–1.3% of adolescents and young adults identify as transgender.[5,10] A school-based survey eliciting gender experiences with scales commonly used at gender identity services suggested that 1.3% of 16–19 year olds had potentially clinically significant gender dysphoria.[11]

Gender identity

Identity is the way one understands, describes and expresses oneself and the reflection of those entities to others. Identity consists of many integrated aspects such as gender, nationality, language, academic and occupational endeavors, and religious and political convictions. It is affected by interpersonal relationships, society and different events throughout the life course.[12] Adolescence is an important period of identity formation and integration.[12,13] Adolescents and young adults establish their identity by actively exploring identity-related choices and making identity commitments in their chosen directions.[12,13]

Gender identity concerns the individual's core sense of being "female", "male" or another gender. The development of gender identity is a complex process affected by multiple factors.[14,15] In the research tradition of gender identity, the broad focus has been on the theme "sex differences", and two major topics have received the most attention: the description and measurement of sex differences and the etiology of these differences.[16] Several theories have been proposed. According to early psychodynamic theories, gender variant behavior was hypothesized to derive from parent–infant interpersonal issues or trauma (see Gray et al[15]). However, these theories have not undergone adequate scientific testing. Gender identity development has mostly been described from the viewpoint of cognitive and social learning theories, which argue that human beings are active constructors of cognitive schemas, including gender, in continuous interaction with the environment.[14] Other theories on processes of gender typing have focused on proximal and distal biological influences, genetic and epigenetic or hormonal and neural mechanisms as well as brain anatomy differences in the etiology of gendered behavior and gender variance.[17–19] There are structural and functional sex differences in the brain, some of them observable across the life span and others only during specific developmental phases. Sex differences in the brain are largely determined by steroid hormone exposure during a perinatal sensitive period that alters subsequent hormonal and non-hormonal responses throughout the lifespan, but they also depend on genes on sex chromosomes. Moreover, there is continuous interaction between genes and experiences, "epigenetics", which changes the expression of genes without any change in the underlying DNA sequence. Research suggests that, for example, early social experiences may act as such epigenetic influence that they ultimately shape lasting sex differences in brain and behavior,[20–22] but a lot more research is needed in this field to obtain solid knowledge relevant for understanding GD.

While the theories proposed in the past have generally been either essentialist or social cognitive/constructivist in nature, researchers today are expanding the focus to include the bio-psycho-social processes that probably occur across development.[14,15,23]

Childhood GD and puberty development

GD in childhood (GDC)[1] describes a feeling of incongruence between the experienced (psychological) gender and the sex

assigned at birth. A corresponding diagnosis is included in the ICD-10.[2] Healthy children vary considerably in their gendered behaviors.[15] The diagnosis of GD in prepubescent children has been widely discussed throughout the history of gender identity research, mainly in terms of weighing the risk of stigmatization against diagnosis as a means of access to publicly funded or insurance-covered health care.[3,24] The prevalence of GDC is not known.[5]

Adolescence is a crucial time for identity and psychosexual development in young people with gender identity concerns.[25] The outcomes of GDC have been discussed in terms of its persistence and desistence. For most children with GDC, whether GD will persist or desist will probably be determined between the ages of 10 and 13 years,[26] although some may need more time.[27] Evidence from the 10 available prospective follow-up studies from childhood to adolescence (reviewed in the study by Ristori and Steensma[28]) indicates that for ~80% of children who meet the criteria for GDC, the GD recedes with puberty. Instead, many of these adolescents will identify as non-heterosexual.[17,29] Steensma et al[26] interviewed adolescents with different outcomes of GDC (persistence or desistance). The adolescents mentioned social environment, the anticipated results of bodily changes and first romantic and/or sexual experiences as central factors in the desistance or persistence of GD.

Treatment of GD intensifying in puberty: the Dutch model

The most commonly used guidelines for the treatment of GD in children and adolescents are those of The Endocrine Society[30] and the Standard of Care from the World Professional Association for Transgender Health,[31] which are based on the so-called Dutch Model protocols published and practiced at the Amsterdam Gender Clinic in the Netherlands.[32]

The Dutch protocol recommends medical treatment if GD intensifies in puberty, while the care for children with GD and their families consists of providing information, psychological support, parental or/and family counseling. In adolescents, medical treatment is recommended at age 12 years and older for those who are in or beyond the early stages (Tanner II–III) of puberty and are still experiencing persistent GD. Puberty suppression with gonadotropin-releasing hormone analogs is part of the protocol for these patients. The purpose of puberty suppression is to relieve the psychological suffering caused by the development of secondary sex characteristics, to give the adolescent time to make a balanced decision regarding whether to undergo actual medical gender-confirming treatment (with cross-sex hormones and surgery) and to make social "passing" in the experienced gender easier. Cross-sex hormones are used for adolescents aged 16 years and older who continue to experience persistent GD. People aged 18 years and older with a diagnosis of GD may undergo SR surgery.[32]

Outcome of and ethical debates around medical interventions for GD in adolescence

The Dutch protocol is largely used, but it has its critics.[33–35] Controversy regarding the use of drugs for puberty touches on fundamental ethical concepts in pediatrics: the best interests of the minor, autonomy and the role of social context. Professionals recognize the distress of young people with GD and feel an urge to treat them. At the same time, most of these professionals have doubts because of the lack of data regarding long-term physical and psychological outcomes.[36,37]

Reports of the outcomes of puberty suppression treatment in adolescents have shown reasonable safety and good outcomes regarding patient satisfaction and psychosocial functioning, but research is still scarce. Nevertheless, puberty suppression is not indicated in a considerable proportion of gender dysphoric minors because of several reasons, for example, severe psychiatric comorbidity, considerable instability of psychosocial support or onset of GD later during puberty and diagnostic uncertainty;[38–40] nevertheless, more follow-up data even from patients who are fulfilling the criteria for "the Dutch model" are still needed.[37]

Psychiatric disorders among adolescents with GD

Descriptive studies of adolescents referred to specialized gender identity services at different centers in Europe and North America have mainly suggested that ~40%–45% of these young people present with clinically significant psychopathology.[38,39,41–50] The lowest figures for psychiatric comorbidity (one-third of the presenting population) were reported in the Netherlands,[41] and the highest (up to three quarters) was reported in Finland and Canada.[39,50] Gender-referred adolescents actually appear to display clinically significant psychopathology to the same extent as adolescents referred to mental health services due to other reasons.[48,50] The most commonly reported disorders are depression and anxiety disorders. Self-harm and suicidal ideation/behavior are also common, whereas conduct disorder and antisocial development do not appear central in this population.

Likewise, community-level information suggests that transgender-identifying youth present four to six times more often with depression and three to four times more often with self-harm and/or suicidal behavior compared with cisgender adolescents.[10,51] Clinical and population data, though scarce, also suggest an overrepresentation of eating pathology among adolescents with GD or transgender identity.[46,52]

An increased prevalence of autism spectrum disorders (ASDs), varying from ~6% to over 20%, has been reported among samples of adolescents referred to gender identity services.[39,42,46,53] This vastly exceeds the estimated prevalence of 0.6%–0.7%[54] in the general population. In comparison, among children and early adolescents with ASDs, gender variance is >7-fold more common than among non-referred controls.[53,55]

Hypotheses to explain this are manifold. The theory of the extreme male brain suggests that individuals with ASD demonstrate an extreme of the typical male pattern of behaviors and cognitions originating from high levels of fetal testosterone. High fetal levels could likewise contribute to GD in natal girls, explaining their male identity and behavior. However, this theory cannot explain the association between ASD and GD in natal boys. Social factors-related hypotheses propose that the social perception and communication difficulties typical of autism could make a child more likely to miss social cues regarding how to conform to gender norms or to identify with the opposite sex when he/she faces difficulties joining the peer group of her/his own sex. Hypotheses focusing on individual psychological characteristics suggest, firstly, that gender could be among the preoccupations or obsessions often seen in ASDs. On the other hand, the development of atypical gender identity in autism could relate to the developmental rigidity typical of autism. Individuals with ASD might not reach normative flexibility in gender development necessary to deal with gender variant feelings, which might lead to the overrepresentation of ASD in GD.[53,56] The suggested causes, however, remain speculative. In a recent study, both boys and girls with GD displayed elevated levels of autistic symptomatology in all subdomains of autism, which did not exclusively support any of the suggested hypotheses.[56] Nevertheless, ASDs pose particular challenges for the diagnosis and treatment of GD in adolescents.

GD and the developmental tasks of adolescence

"Developmental tasks" refer to the normative developmental milestones that should be reached during a given developmental stage.[57,58] They arise from interactions among physical development, personal attributes and societal expectations. Favorable completion of the developmental tasks of a given stage is a prerequisite for success in the subsequent stages. The developmental tasks of adolescence were first formulated by Havighurst[57] and comprise accepting one's body, adopting a masculine or feminine social role, achieving emotional independence from parents, developing close relationships with peers of the same and opposite genders, preparing for an occupation, preparing for marriage and family life, establishing a personal value or an ethical system and achieving socially responsible behavior. Although puberty now occurs earlier and the transition to adulthood occurs later than they did when these developmental tasks were initially proposed, they remain relevant.[58] The relationship with one's own body and the acquisition of a gendered social role – not necessarily binary – are by definition challenging for adolescents with GD. In the following sections, we discuss the available information on GD/transgender identity and the other developmental tasks of adolescence.

GD in adolescence and relationships with parents

Parents of adolescents with GD and/or transgender identity may face special challenges that are shaped by a variety of factors, such as ethnicity, religious background, social class and the prevailing attitudes in their community and society.[59,60] These challenges likely shape the support that a nonconforming adolescent can receive. Adverse parental reactions toward an adolescent's gender nonconformity have been noted as a special risk,[61] but parents of sexual- and gender-minority offspring have also reported particular positive aspects of being a parent in this situation, such as personal growth, unconditional love, activism, social connection and closer relationships.[62] However, few studies have empirically explored the parental reactions and support among youth with GD and/or transgender identity.

In a Canadian community study of transgender-identifying youth,[63] of those who had disclosed their gender identity to their parents, 34% considered their parents "very" supportive and 25% considered their parents "somewhat" supportive. Forty-two percent reported that their parents were "not very" or "not at all" supportive. However, the study was based on a nonrandom sample and solely adolescent self-reports, so findings need to be interpreted with caution and causalities cannot be concluded. Strong perceived parental support was, nevertheless, associated with many positive mental health outcomes. Lack of parental support was associated

with inadequate housing and homelessness in addition to negative psychological outcomes. Better parental support has also been associated with fewer risk-taking sexual behaviors among transgender youth.[64]

In a community study of trans female adolescents and young adults,[65] more than half of the participants reported that their parents supported their gender identity, showed their support in many ways and believed the respondent could have a happy future as a trans adult. However, approximately two in five respondents had not experienced parental acceptance. Parental acceptance was associated with perceiving parents as the primary source of social support.

In a school-based survey[51] transgender-identifying adolescents felt less often (odds Ratio 0.3) than their cis-gender peers that at least one parent cared for them.

Studies of clinically referred gender dysphoric youth have rarely addressed parent-related issues. Simons et al[66] reported that in a clinical sample of adolescents with GD, parental support was significantly associated with higher life satisfaction, lower perceived burden of being transgender and fewer depressive symptoms. In a Finnish study comparing childhood gender identity in community and clinical samples, a smaller proportion of adolescents with GD than of non-referred adolescents in the population agreed with the statement "I always felt that my parents cared fore me."[11] It was also noticed that the clinically referred adolescents with GD less commonly lived with both their parents than the adolescents in the normal population (48% vs. 78%).[67] In British and Spanish samples of gender-referred adolescents, parental divorce was observed in the background of approximately three in five participants, but the authors did not compare this finding with a corresponding rate in the general population.[46,49]

Gender nonconformance and peer relationships in adolescence

During adolescence, peer relationships are critical for psychological well-being.[68,69] Peer relationships also shape development, including aspects of gender identity consolidation.[70] Loneliness and social isolation from peer relationships is associated with developmental difficulties and impaired mental health.[71,72] An important peer network-related risk factor is bullying.[73]

Observations in referred samples of adolescents with GD suggest considerable peer relationship difficulties. In both the UK[46] and in Finland,[39] approximately half of adolescents who presented at a specialized gender identity service reported significant experiences of being bullied. In the Finland study, 45% of the referred adolescents also had a history of marked periods of social isolation in childhood and/or adolescence. In the Netherlands and in Canada, self-, parent and teacher ratings indicated poorer peer relationships among adolescents referred for GD than in the same-aged population[47,48] and poor peer relationships were an important correlate of mental health problems in this group. Similarly, in another Canadian comparison among gender-referred, mental health-referred and general population adolescents bullying was reported by the GD group more commonly than by population controls, and to the same extent as by those referred due to mental health issues. Gender-related bullying was most common among the GD group.[74]

On the population level, Clark et al[51] found that transgender-identifying adolescents had 4.5-fold increased odds of being bullied and were approximately twice as likely to report being afraid for their personal safety, having been in a serious physical fight and having been hit or otherwise harmed by others, compared with their cisgender-identifying peers. They also less commonly felt that their friends cared about them and that school was okay.

Gender-nonconforming behavior is characteristic of both sexual- and gender-minority youth and has been associated with an increased likelihood of experiencing bullying and harassment in peer groups.[75,76] Adolescents with GD likely represent the extreme end of gender nonconformity, and this may strongly contribute to their experiences of being bullied. Bullying and stigmatization have also been suggested to (partially) mediate the association between gender nonconformity and lower mental well-being across adolescence.[74,77–79]

However, not all the difficulties the gender dysphoric adolescents face in peer relationships can be explained by gender expression-related victimization or discrimination. In the Finnish clinical sample, of the gender identity-referred adolescents who had experienced severe bullying at school, three quarters had been bullied before they ever questioned their gender. Likewise, three-quarters of them reported that the bullying had not been related to gender expression or sexual identity, but to other factors such as not being slim, being successful at school or having unfashionable hobbies and interests.[39] Bullying is a severe problem regardless of the reported reasons for it, but it is important to acknowledge that adolescents who develop GD also have unrelated difficulties that may need attention.

GD, transgender identity and sexuality in adolescence

Sexual orientation and gender identity are different entities, and transgender people present with a variety of sexual orientations. Nevertheless, sexual orientation has long been used

to subtype GD/transsexualism.[80] During adolescence, the different facets of sexual orientation – attraction, behavior and identity – may still be developing. It may be more important to determine whether adolescents with GD or transgender identity display developmentally appropriate and favorable involvement in romantic and erotic relationships.

In adolescence, sexual development accelerates. Young people's experiences of a maturing and changing body, sexuality and their developing gender identity affect intrapersonal, interpersonal and societal interactions.[81] In Western countries, between one-tenth and one-third of adolescents first experience sexual intercourse by the age of 15, and the vast majority experience it by age 20.[82,83] Various practices of kissing and petting typically precede first sexual intercourse by several years. Early sexual activity has been viewed as a problem behavior associated with risky sexual behaviors, psychosocial difficulties and emotional and behavioral disorders.[82,83] In contrast, in the late stages of adolescent development, a lack of experiences might suggest developmental difficulties.

GD and/or transgender identification could be expected to be associated with delayed sexual development, given that it is the sexual body, in particular, that is the source of distress in GD and that differing from the mainstream may increase the adolescent's risk of problems in social relationships, including dating, and sexual encounters. Sexual- and gender-minority adolescents may also have a reduced availability of potential partners and increased challenges in finding potential partners than their heterosexual peers.[84] However, developmental challenges have also been associated with premature and risky sexual behavior.[82,85] Adolescents with GD and/or transgender identification could engage in risky sexual behaviors due to identity experiments or because associated mental health problems could increase their search for comfort in intimacy or decrease their self-protection skills.

To the best of our knowledge, the only study focusing on the sexual experiences of treatment-seeking adolescents with GD is that of Bungener et al[86] from the Netherlands. They compared the sexual experiences of 137 transsexual adolescents (mean [SD] age 14.69 [2.2] years) with those of a same-aged adolescent population. Transsexual adolescents had fewer sexual experiences than the same-aged population in all areas measured (falling in love, romantic relationships, kissing, petting, intercourse). However, a majority of the transsexual adolescents had fallen in love and approximately half had been involved in romantic relationships. One quarter had experienced petting while undressed, and 5% had experienced sexual intercourse. Fewer transsexual adolescents than the adolescents in the same-aged population (24% vs. 48%) valued sex as important. In a descriptive study of clinically presenting adolescents with GD in the USA,[45] nearly half of the respondents (mean [SD] age 19.2 [2.9] years) reported being sexually active.

Some population studies provide information regarding transgender identity in adolescence and aspects of sexual development. Korchmaros et al[84] compared the romantic relationships of lesbian, gay, bisexual, transgender and questioning (LGBTQ) adolescents and those of adolescents with mainstream sexual and gender identities. Contrary to expectations, the LGBTQ adolescents were more experienced with romantic relationships and more active in initiating relationships both online and offline. Results were not reported separately for the transgender group. Robinson and Espelage[87] reported that LGBTQ adolescents were more likely to display risky sexual behaviors than same-aged non-LGBTQ youth. However, in more detailed analyses, the risk was associated with homosexual/bisexual orientation and not with transgender identity. Veale et al[88] set out to study pregnancy involvement among transgender youth and the health correlates of this involvement. In a large (n=923) sample of transgender-identifying youth, 540 responded to the pregnancy involvement item. Almost 5% of Canadian transgender adolescents had ever been pregnant or impregnated a partner; approximately the same proportion as their same-aged peers. Those with a history of pregnancy involvement were also more likely to have a history of sexually transmitted disease, but they did not differ from the rest of the transgender youth in terms of hormone use, living in the felt gender, self-reported mental health and level of social support.

Sexual harassment is a common problem among adolescent populations.[89] Transgender-identifying adolescents appear to be at the greatest risk of sexual harassment and to experience the greatest distress due to it.[89] Sexual harassment is suggested to function to maintain heteronormativity, which transgender adolescents likely challenge. Their perception of sexual harassment as more distressing compared with other adolescents could be due to harsher harassment, increased vulnerability due to uncertainty about self, or fear.[89]

Similarly, in a large school-based survey study on teen dating violence,[90] the few transgender-identifying youth in the sample reported the highest victimization rates for physical dating violence, psychological dating abuse, cyber dating abuse and sexual coercion. Differences from cisgender adolescents varied from 2- to 7-fold for the different forms of violence. However, the transgender-identifying youth also reported the highest rates of perpetrating dating violence. Minority stress theory[91] posits that the chronic stressors that

minorities experience (e.g., gender-based discrimination) shape their coping mechanisms (such as substance use, aggression) and lead to adverse psychosocial and health outcomes. The particular vulnerability to perpetrating dating violence observed among transgender adolescents by Dank et al[90] could be understood through minority stress theory, but more research is needed.

Transgender adolescents and young adults, particularly trans females, are at a disproportionately high risk of contracting human immunodeficiency virus and other sexually transmitted diseases.[79,92] The risk of unprotected sex in this population has been associated with sex work and drug use, which are further associated with rejection, stigma and discrimination.[79,92] Of the studies of referred samples, only one addressed sex work.[45] In that sample, 6% of the referred adolescents reported engaging in the trading of sex.

Sexual education is an important way to promote positive and responsible sexual behaviors in youth. The planned curricula and practical applications likely vary widely across countries and schools. Sexual- and gender-minority youth were found to desire minority-inclusive sexual education in a study by Gowen and Wingez-Yanez.[93] The sexual- and gender-minority youth felt that the sexual education that was offered isolated them by silencing them, adopting a heterocentric perspective and pathologizing minorities. Reflecting on the available sexual education in light of these findings is appropriate for all educators.

Preparing for occupation: academic performance and socioeconomic status

To the best of our knowledge, research has not specifically focused on academic performance and the progression to work life among adolescents with GD, but given the burden of psychiatric comorbidities among gender-referred youth, special needs regarding education are likely to exist.

Aspects of social relationships are relevant to well-being in school, school performance and pathways to occupation. Transgender youth have been reported to experience bullying and discrimination in schools, not only by peers but even by teachers; consequently, they perceive schools as unsafe places, which again increases the risk of non-attendance and poorer results.[75,94] Gender- and sexuality-related victimization may impair academic performance through, for example, decreased motivation, concentration and self-efficacy and the resulting school avoidance and harmful coping strategies.[94,95] Nevertheless, being "out" at school improves self-esteem among gender- and sexual-minority youth and increases their well-being, which can have a positive impact on academic performance.[94]

School dropout is strongly linked to social exclusion. School dropout was associated with high masculinity in girls and low masculinity combined with high femininity in boys in a study of late-adolescent school dropouts and attenders in the Netherlands.[96] The authors suggested that such deviation from gender norms increases the risk of unpopularity among peers, which again predisposes individuals toward school dropout. However, school dropout was also associated with very masculine attitudes and self-evaluations among boys. The role of gendered behaviors, attitudes and experiences in school adjustment and academic performance deserves further research.

In Clark et al's[51] school-based survey, adolescents reporting non-cisgender identity came disproportionately often from families with high socioeconomic deprivation and less often felt that their family got along. Any explanation for this remains unknown; however, young people are likely to stay in the same socioeconomic position as their parents.[97] Jacob and Cox[98] also pinpointed transgender people's greater risk of having a disadvantaged socioeconomic status (in the USA), associating this with increased unemployment, and employment in low-paid jobs, because of stigmatization.

Why the increase in referrals?

Zucker et al[99] observed an increase in the number of adolescents presenting at gender identity services in the early 2000s. Since then, several gender identity services for minors from across Western countries have reported increases.[8,9,42,49] Simultaneously, the earlier overrepresentation of natal boys has equaled or turned to overrepresentation of natal girls.[9] Natal girls now comprise from half[49] to ~90%[39] of clinical adolescent samples. The reasons for these changes are not known. The increase in referrals could be attributable to enhanced provision of services, or the threshold for seeking help may now be lower due to increased knowledge and improved societal acceptance. Aitken et al,[9] however, did not find evidence supporting a lowered threshold to gender identity services. Sociocultural features related to what kind of identities are available for whom, and sex-related differences of pressure to conform may play a role.

Comments

Research regarding the clinical treatment of adolescents with GD has mainly focused on childhood-onset GD that intensifies during puberty, and the Dutch treatment protocol is also tailored for this group. There is little empirical knowledge

regarding young people who experience their first signs of GD in adolescence, well after the onset of puberty, especially regarding biological girls.[50,100] Among a treatment-seeking sample in the UK, 18% experienced their first feelings of GD in adolescence[46] compared with approximately two-thirds of the Finnish sample,[39] and for the majority of adolescent-onset cases, GD presented in the context of severe mental disorders and general identity confusion. In such situations, appropriate treatment for psychiatric comorbidities may be warranted before conclusions regarding gender identity can be drawn. Gender-referred adolescents actually display psychopathology to the same extent as mental health–referred youth.[48,50] In a nationwide long-term follow-up study of adult cases, psychiatric morbidity, suicide attempts and suicide mortality persisted as elevated after juridical and medical SR.[101]

Emerging discussions raise concern for post-pubertally abruptly emerging cross-gender identification ("rapid onset"), particularly among biological girls, suggesting a role for intensive media influences and generous group validation as shaping the understanding of, and giving new meanings to, the body discomfort common among female adolescents at large.[100] The persistence of increasing adolescent-onset transgender identification is not known.[5,100]

More empirical research is needed regarding virtually all aspects of GD in adolescence to create treatment approaches that optimize these young people's future psychosocial health and well-being. It seems unlikely that all the psychopathology observed in the referred samples is secondary to gender identity issues and would resolve with hormonal and later surgical treatments. There is still no clear consensus regarding hormonal treatment for adolescents because long-term data are unavailable;[36] actually, only one long-term follow up has been carried out, with a highly selected intervention group and an at baseline non-comparable comparison group.[102]

An affirmative approach[103] is increasingly implemented in the health care of gender nonconforming children. This includes, based on a comprehensive psychological and psychosocial assessment, work with the children and their families and schools to support the gender-nonconforming minors to express themselves in a way that feels most comfortable for them. With the starting point that gender presentations are fluid and changing over time, gender variant children need to be allowed to freely explore a range of gender identities and expressions. A debate concerns whether or not a prepubertal child should be allowed to completely transition to live in other than birth gender. Concerns include that childhood transition may be forcing adolescents to proceed to biomedical interventions, as stepping back may be psychologically troublesome, even though identity development has taken a new direction.[28,104]

The etiology of gender incongruence remains unknown. Gender identity differentiation does not occur in a psychosocial vacuum; instead, research in the field suggests that the developmental course is influenced by numerous psychosocial factors, likely in continuous interaction with biological factors.[23,105] Gray et al[15] noted that the general narrative in the research literature concerning gender variation among children focuses on gender "atypical" behavior and deviation from "normative patterns", thus viewing gender in a binary way instead of as a wider spectrum of (healthy) identities, personalities and behaviors among children. This is surely relevant for adolescents as well. These authors also requested a shift in research paradigms away from the study of outcomes of sexuality and gender identity and the child/adolescent in isolation toward outcomes of adjustment and the child/adolescent in contexts that affect adjustment. Along with further discussions of the best treatment interventions, it is relevant to attempt to contribute to societal attitudes that enable children and adolescents with gender variance to express themselves and successfully complete the developmental tasks common to all, independent of gender.

Disclosure

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Sex- and Gender-specific Observations and Implications for COVID-19

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Disclaimer: Due to the rapidly evolving nature of this outbreak, and in the interests of rapid dissemination of reliable, actionable information, this paper went through expedited peer review. Additionally, information should be considered current only at the time of publication and may evolve as the science develops. On February 11, 2020, the World Health Organization renamed the virus COVID-19.

This is a critical time for medicine. As we observe the exponential rise in the number of individuals in the United States (US) who are infected with COVID-19, we try to prepare. Those in the front lines are trying to protect themselves and their patients with the daily ration of personal protective equipment and ventilation assistive equipment. Many individuals are racing against time to develop the needed novel treatments and vaccines. Public health officials work with what little information is known in order to make effective recommendations for prevention. However, at this pivotal time in history where every detail obtained by US health officials could be lifesaving, we are leaving out vital information.

Descriptive and observational data from Wuhan, China, note that the majority (51%-66.7%) of affected patients have been male. In addition, male sex was an independent risk factor associated with refractory disease and death (2.8% death rate for men vs 1.7% for female).[1,2] Currently, men represent 58% of COVID-19 infected patients in Italy and 70% of COVID-related deaths.[3] As coronavirus cases and deaths in the US continue to soar, sex-specific, comprehensive data with regard to US patients is not yet available.

Sex- and gender-based medicine (SGBM) incorporates how biological *sex* and the sociocultural aspects of *gender* affect health and illness. It acknowledges the interrelationship between sex and gender on health outcomes and promotes consideration of this variable in both research and clinical practice. SGBM has demonstrated significant evidence-based impact on cardiovascular disease, stroke, sports medicine, and pain management, just to name a few

Sex and gender differences have been observed in infectious diseases previously. On a broad and critical scale, Nasir et al demonstrated that males with all-cause infectious sepsis have a 70% greater mortality than their female counterparts. Likewise, respiratory infection-specific epidemiological data from recent SARS (2003) and MERS (2012) outbreaks demonstrated a significantly higher case fatality rate in males as compared to females, 21.9% vs 13.2%.[4,5]

Sex-specific Factors

Is the biological male more susceptible to an increased severity of infection? Or does the biological woman have natural protection against these viruses? In a 2017 *BMJ* article, Dr. Kyle Sue demonstrated the effect of sex hormones, estrogen and testosterone, on immune system response and engagement, resulting in a less robust immunologic response in males and subsequent increased morbidity and mortality from viral respiratory illnesses.[6] In addition, the X chromosome carries the largest number of immune-related genes in the human genome, perhaps also contributing to female's superior immune response (as well as a female preponderance in autoimmune diseases).[7]

Angiotensin-converting enzyme 2 (ACE2) and its role in viral transmission and associated morbidity has also been a topic of recent COVID-19 associated discussion. ACE2 receptors on pulmonary endothelium serve as a main entry point for coronavirus. Several previous animal models have demonstrated increased ACE2 activity in the male or ovariectomized model, suggesting a sex hormone influence.[8] The gene for the ACE2 receptor is also, interestingly, on the X chromosome.[9]

Gender-specific Factors

Behavioral and cultural variables have also influenced current COVID-19 epidemiology. Smoking in particular has

been implicated as a significant contributor to disease severity, and gender-specific patterns are quite apparent here. The smoking rate in China is much higher in men than in women (288 million men vs 12.6 million women; 2018 data).[10] Likewise, in Italy, men are more likely to smoke than women at any age (1.12x to 1.7x, depending on age cohort; 2018 data).[11] Similar gender-specific trends are also present in the US, where 17.6% of smokers are men as compared to 13.6% of women.[12]

In addition, as the traditional caregivers and coordinators of care for their loved ones, women, particularly working mothers, tend to spend more time than men focused on medical issues related to both their own healthcare and that of their families.[13] In general, men are more likely to engage in health-related risks which, even prior to the COVID-19 pandemic, has been shown to result in higher rates of injury and disease.[14] Suen et al demonstrated in 2019 that being a middle-aged female was a protective factor with regard to hand hygiene knowledge and practice.[15]

Population Health Research Capsule

What do we already know about this issue?
COVID-19 represents an unparalleled public health crisis. Like many other infectious diseases, sex and gender differences in health outcomes have already been globally observed.

What was the research question?
We sought to summarize and explain known COVID-19-related sex and gender differences.

What was the major finding of the study?
Sex and gender differences are having significant impacts on current COVID-19 health outcomes.

How does this improve population health?
This perspective brings attention to the importance of sex and gender, specifically as they impact current clinical management and research during the COVID-19 pandemic.

Implications for COVID-19 Management

As clinical researchers and pharmaceutical companies race to find an effective treatment strategy or vaccine for COVID-19, no sex- or gender-specific recommendations have been released with regard to the care and management of individuals affected by the novel coronavirus. Appreciating the weight of known sex- and gender-specific epidemiologic observations thus far, however, will be an important highlight of the information gathered to date. This, combined with what is already known about sex- and gender-based pulmonary and infectious disease pathology, may lead to important treatment breakthroughs that consider the sex and/or gender of patient in the comprehensive management plan.

In addition, the current pandemic weighs heavily on emotional wellness along with physical health. COVID-19 has also released a contagion of fear, anxiety, and stigma that will have implications for downstream mental health effects including post-traumatic stress disorder (PTSD). In general, the prevalence of PTSD has been shown to be substantially higher in women.[16] This has been re-substantiated in the setting of the COVID-19 outbreak in Wuhan, China, where women scored significantly higher on the PCL-5 (DSM-5 self-report measure for PTSD) than men, including higher rates of re-experiencing and negative alterations in cognition or mood.[17] Early recognition and effective treatment can ameliorate the burden of PTSD on both the individual and society, particularly for women who have been shown to have a modest advantage with regard to treatment response.[18]

Future Considerations

Since 2016, the NIH has required inclusion of sex as a biological variable (SABV) in the study design for funded research.[19] Recognizing the weight these variables play in disease outcome should result in universal adoption of SABV as scientists develop and engage in COVID-19 research. While men appear to be disproportionately affected and at risk for COVID-19 infection and associated morbidity, researchers should avoid the slippery slope of the traditional male-dominant test and analysis approach.

When considering pharmaceutical therapy advances, several previous studies have established that women are much more likely to experience adverse drug reactions (ADR) than men.[20] In fact, historically the majority of drugs recalled from the market were done so due to serious ADRs reported by women, quite often because they were never tested on women during clinical trials. Several sex-specific pharmacokinetic and pharmacodynamic differences have been well documented.[21]

Yes, time is of the essence right now; however, COVID-19 does not get a "pass" in considering sex and gender when gathering data or testing treatments. Sex and gender have already proven to be crucial variables in the short history of COVID-19; they will continue to be factors of marked importance. Making healthcare providers and researchers aware of their impact in real time will be crucial to the integration of susceptibility profiles and improving outcomes during an active public health crisis.

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Considering how biological sex impacts immune responses and COVID-19 outcomes

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Abstract | A male bias in mortality has emerged in the COVID-19 pandemic, which is consistent with the pathogenesis of other viral infections. Biological sex differences may manifest themselves in susceptibility to infection, early pathogenesis, innate viral control, adaptive immune responses or the balance of inflammation and tissue repair in the resolution of infection. We discuss available sex-disaggregated epidemiological data from the COVID-19 pandemic, introduce sex-differential features of immunity and highlight potential sex differences underlying COVID-19 severity. We propose that sex differences in immunopathogenesis will inform mechanisms of COVID-19, identify points for therapeutic intervention and improve vaccine design and increase vaccine efficacy.

The COVID-19 pandemic, caused by the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in millions of infections and hundreds of thousands of deaths worldwide. Human biological sex plays a fundamental role in heterogeneous COVID-19 outcomes. Sex, defined as male, female or intersex on the basis of sex chromosome complement, reproductive tissues (ovaries or testes) and sex steroid hormone (oestrogen, progesterone and testosterone) concentrations, is a multidimensional biological characteristic that shapes infectious disease pathogenesis. We discuss how sex differences in basic molecular and cellular mechanisms can be leveraged to define the immune response to infection with SARS-CoV-2.

Sex differences in COVID-19 severity

The precise drivers of death, regardless of sex, in COVID-19 remain unknown. There appears to be a subset of patients in whom high levels of dysregulated inflammation lead to severe multisystem organ pathology[1,2]. A postviral inflammatory syndrome has also emerged in children with COVID-19 (REFS[3,4]). As a result, research on therapeutics has focused on both antiviral and immunomodulatory pathways[2,5] with the goal of achieving an optimized balance in immune response induction and resolution. Unfortunately, most studies fail to consider the sex of the patients, which may mask therapeutic targets.

Evidence of sex differences in COVID-19 severity emerged in China, where hospital admissions and mortality were higher among males than females[6–8]. In South Korea, where community testing was widespread, females represented ~60% of those testing positive for SARS-CoV-2, suggesting that females acquire infection, despite having a lower case fatality rate (CFR)[9,10]. In the United States, where testing was prioritized for people with symptomatic disease, the diagnosis rates were similar in males and females, but males had 1.5 times higher mortality[11].

A male bias in COVID-19 mortality is currently reported in 37 of the 38 countries that have provided sex-disaggregated data (FIG. 1a). Our analyses show that the average male CFR across 38 countries is 1.7 times higher than the average female CFR ($P < 0.0001$) (male CFR 7.3 (95% CI 5.4–9.2); female CFR 4.4 (95% CI 3.4–5.5)), which is consistent with other reports[12,13]. There is increased risk of death for both sexes with advancing age, but at all ages above 30 years males have a significantly higher risk of death than females ($P < 0.05$) (FIG. 1b). A male predominance of deaths from COVID-19 is consistent with what was observed in the prior SARS[14,15] and Middle East respiratory syndrome (MERS)[16] epidemics (caused by SARS-CoV and MERS-CoV, respectively). Although gender-related social factors, including smoking, health care-seeking behaviours and some co-morbid conditions, may impact the outcomes of COVID-19 (REFS[6,17]) and contribute to male–female differences in disease severity, the cross-cultural emergence of increased risk of death for males points to biological risk determinants. In animal models of SARS-CoV infection, differences in mortality between male and female mice were observed and were attributed to steroid hormones[18]. Multiple dimensions of biological sex, including sex steroids, sex chromosomes and genomic and epigenetic differences between males and females, impact immune responses[19–26] and may affect responses to SARS-CoV-2 infection[27].

Ageing, sex and COVID-19

Although advancing age is associated with greater risk of death in both sexes, the male bias remains evident (FIG. 1b). An analysis of COVID-19 data from Italy, Spain, Germany, Switzerland, Belgium and Norway reveals that among all age groups older than 20 years, fatality rates are greater for males than females[28]. By contrast, male–female differences in the rate of confirmed SARS-CoV-2 infections are age dependent in all countries, being greater among females aged between 10 and 50 years and greater among males before the age of 10 years and after the age of 50 years[28]. The age-related male–female differences in confirmed cases of SARS-CoV-2 infections are consistent with reported confirmed cases of seasonal and pandemic influenza A virus infections in Australia and Japan[29,30]. We interpret these data to suggest that biological sex differences contribute to male-biased death, but gender-associated risk of exposure may affect rates of infection differently for males and females.

With a focus on biology, the impact of age on susceptibility to severe COVID-19 needs to be parsed, with both immunosenescence and dysregulation of innate immune responses as potential

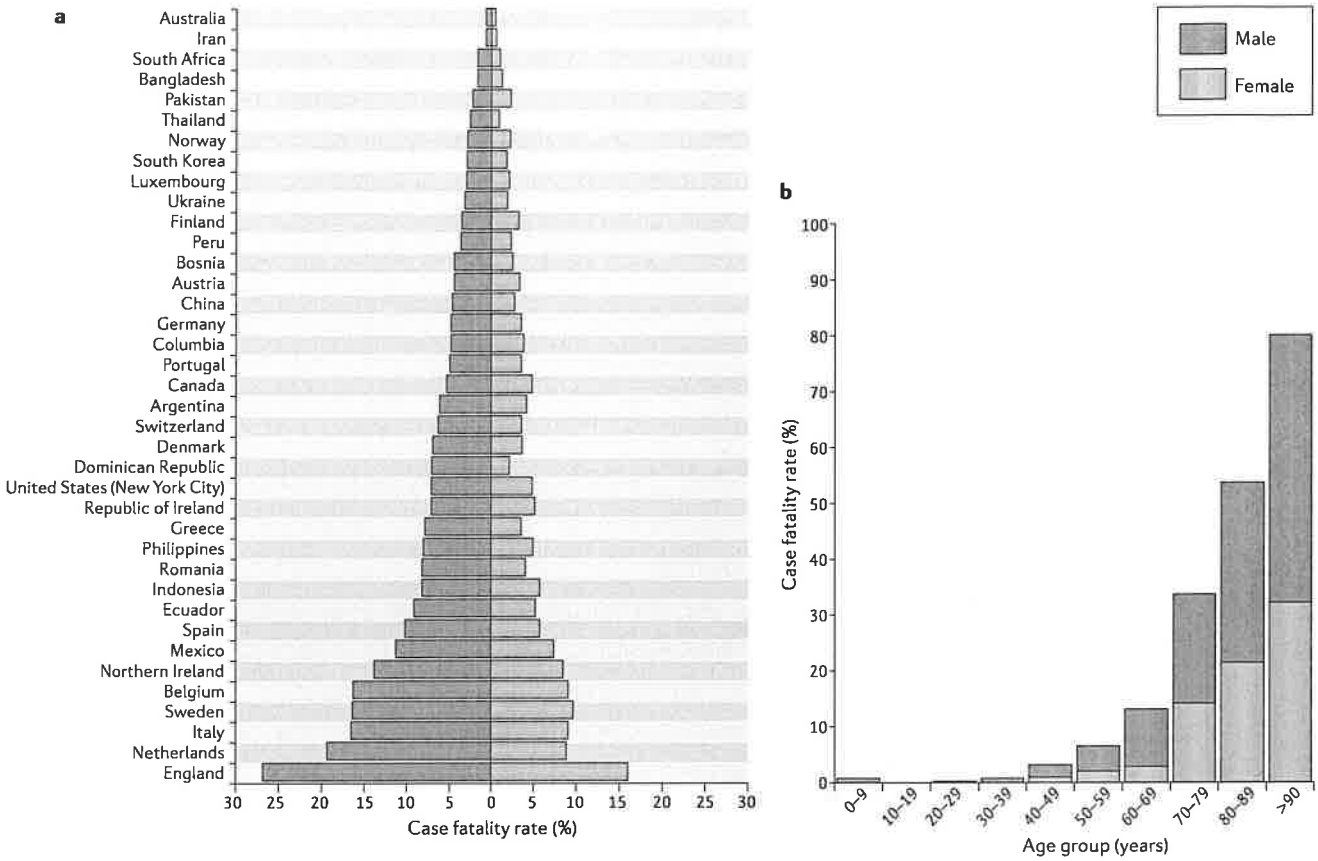

Fig. 1 | **Comparative analyses of COVID-19 case fatality rates by country, sex and age. a** | COVID-19 case fatality rates (CFRs) for males and females across 38 countries or regions reporting sex-disaggregated data on COVID-19 cases and deaths. CFR was calculated as the total number of deaths divided by the total number of cases for each sex multiplied by 100. The male CFR is higher than the female CFR in 37 of the 38 regions, with an average male CFR 1.7 times greater than the average female CFR ($P < 0.0001$, Wilcoxon signed rank test). **b** | Average COVID-19 CFRs for males and females stratified by age. The data represent 12 countries currently reporting sex- and age-disaggregated data on COVID-19 cases and deaths (Australia, Columbia, Denmark, Italy, Mexico, Norway, Pakistan, Philippines, Portugal, Spain, Switzerland and England). The COVID-19 CFR increases for both sexes with advancing age, but males have a significantly higher CFR than females at all ages from 30 years ($P < 0.05$, Wilcoxon signed rank test). The data were obtained from Global Health 50/50 and official government websites of each respective country on 7 May and 8 May 2020. For more information on the data source for a specific country, please contact the corresponding author.

mechanisms[31,32]. Biological sex differentially affects ageing of the immune system[33], in part through changing concentrations of sex steroids[34]. In addition to reduced concentrations of sex steroids, an age-related mosaic loss of chromosome Y in leukocytes can alter transcriptional regulation of immunoregulatory genes[35]. Whether sex differences in the genomic signatures of aged immune cells translate to functional differences in the response to SARS-CoV-2 infection requires attention.

Sex differences in immune responses

Biological sex affects innate and adaptive immune responses to self and foreign antigens, resulting in sex differences in autoimmunity as well as in responses to infections and vaccines[36,37]. Immune cell subsets have sex-specific patterns of gene expression, with most differentially expressed genes found on autosomes, demonstrating sex-specific regulation of shared genetic material[26]. The sex chromosomes also directly contribute. Males are at higher risk of diseases caused by deleterious X-linked alleles. Incomplete inactivation of immunoregulatory genes on the X chromosome can also occur in females, which results in a gene dosage imbalance between sexes[38,39]. Incomplete X chromosome inactivation has been implicated in female-biased autoimmune diseases[40] and in vaccine efficacy[41]. The Y chromosome has immunoregulatory function, broadly impacting immune transcriptional profiles linked to autoimmune disease[42] and impacting outcomes of influenza virus and coxsackie virus infection in animals[43,44]. Sex-specific features of epigenomic organization also dictate differential availability of transcriptional targets[21,45]. Superimposed on these genomic elements is the direct effect of sex steroid exposure. Oestrogens[46,47], progesterone[48–52] and testosterone[53] have direct effects on immune cell function that are driven by the signalling of these hormones through their respective cellular receptors. The variation in sex steroid concentrations that occurs over the life course contributes to differences in immune profiles and disease susceptibility patterns at different ages[20,52]. Consistent with this variation, both sex and age contribute to unique transcriptional signatures of immune cells both at the baseline and after exposure to immunostimulants[19,21,22]. The summative effect is a sex-specific transcriptional regulatory network of genetic variants, epigenetic modifications, transcription factors and sex steroids that leads to a functional difference

in the immune response[35,54]. FIGURE 2 highlights intersections between SARS-CoV-2 infection and sources of sex bias in pathophysiology that warrant further investigation.

Sex bias in SARS-CoV-2 infection

Virus entry receptors. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as an entry receptor, with virus entry enhanced by cellular transmembrane serine protease 2 (TMPRSS2), which primes the spike protein of the virus[55,56]. *ACE2* is an X chromosome-encoded gene that is downregulated by oestrogens[57] and exhibits tissue-specific expression patterns[39]. Differences in *ACE2* expression may be driven by sex-differential expression of *ACE2* variants[58–60]. ACE2 is associated with interferon gene expression[61,62], which in turn shows sex-specific regulation. The cell-intrinsic regulation of ACE2 expression may change with age, in response to changing levels of sex steroids, or following viral challenge. TMPRSS2 is regulated by androgen receptor signalling in prostate cells[63]. Initial investigations have not demonstrated a significant difference in *TMPRSS2* mRNA expression in lung tissue from males and females, but it is unknown whether androgens may impact expression in the setting of infection with SARS-CoV-2 (REFS[63,64]) or whether the level of expression has an impact on SARS-CoV-2 burden. Further research is needed to determine whether sex-biased expression of *ACE2*, coupled with the regulation of *TMPRSS2* by androgens, increases SARS-CoV-2 susceptibility of males compared with females.

Interferons. Innate sensing of viruses, production of interferons and activation of the inflammasome are the first line of defence against viruses[65]. In the case of SARS-CoV-2, where there is no pre-existing adaptive immune memory, the success of this early antiviral response may be a determinant of disease outcome. Innate sensing of viral RNA by the pattern-recognition receptor Toll-like receptor 7 (TLR7) is sex biased, as *TLR7* escapes X chromosome inactivation, resulting in greater expression in female immune cells; this has also been linked to sex differences in autoimmunity[10,66] and vaccine efficacy[11]. There is greater production of interferon-α (IFNα) from plasmacytoid dendritic cells from adult females than from adult males[67,68], an effect modulated by sex steroids[69–71]. In animal models of SARS-CoV infection, pretreatment with pegylated IFNα was associated with protection of lung tissue[72] but without consideration of biological sex. In SARS-CoV-2, emerging data suggest that there is aberrant activation of interferon responses but preserved chemokine signalling, which has been postulated to contribute to immunopathology[73]. Studies are needed

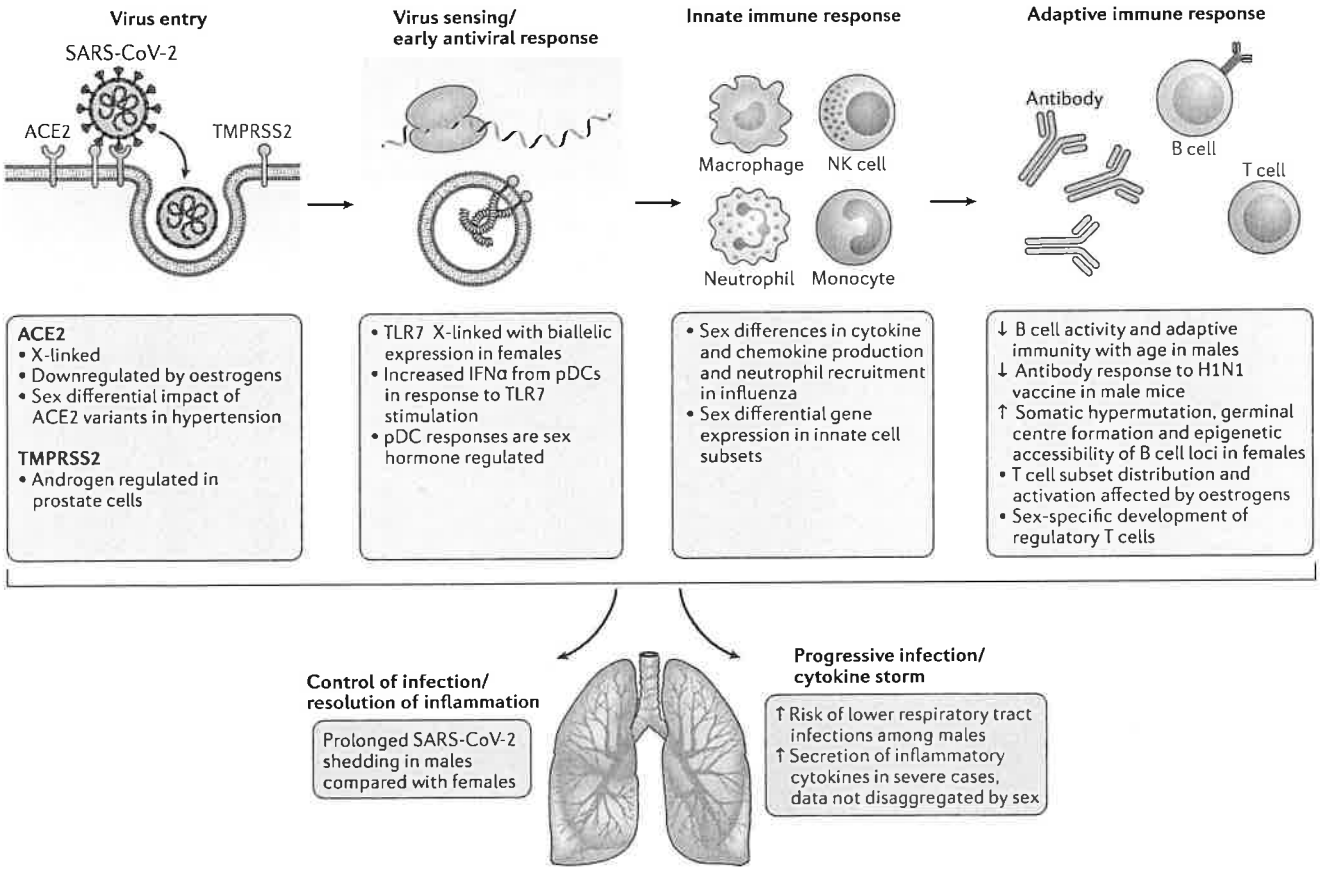

Fig. 2 | **Known sex differences that may impact immune responses to SARS-CoV-2 and COVID-19 progression.** An illustrative summary of the sequence of events in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the associated immune responses. Broadly speaking (from left to right), there are the initial steps of virus entry, innate recognition of the virus with activation of antiviral programmes, the recruitment of innate immune cells and induction of an adaptive immune response. These major steps culminate either in successful control of infection and pathogen elimination or in a pathological inflammatory state. Sex differences that may be operative at multiple points along these pathways are indicated in the blue boxes. ACE2, angiotensin-converting enzyme 2; H1N1, H1N1 influenza virus; IFNα, interferon-α; NK, natural killer; pDC, plasmacytoid dendritic cell; TLR7, Toll-like receptor 7; TMPRSS2, transmembrane protease serine 2.

to determine whether differences in the magnitude or kinetics of the interferon response may contribute to a sex bias in the early control or severity of SARS-CoV-2 infection and may inform considerations of interferons as therapies for COVID-19 (REF.[74]). Early data suggest that male sex may be associated with a longer duration of viral detection, even within families[75,76], raising the question of whether females have more efficient clearance of the virus. The rate of virus clearance will need to be assessed in evaluating the efficacy of innate and adaptive immune responses.

Adaptive immunity. Females generally mount greater antibody responses to viral infection and vaccination, albeit with higher levels of autoreactivity[77]. The mechanisms for sex differences in antibody production include oestrogenic enhancement of somatic hypermutation[78], less stringent selection against autoreactive B cells[77,79–82] and sex differences in germinal centre formation[83] and in the epigenetic accessibility of B cell loci[21]. It is still unknown whether sex has an impact on antibody generation in SARS-CoV-2 infection. Early studies suggest that titres of antibodies to some viral epitopes are higher in patients with severe COVID-19 and that seroconversion may not be tightly linked to declining virus titres[84,85]. Ongoing studies evaluating the infusion of convalescent serum may provide answers as to the protective capacity of these antibodies[86], but these studies are currently not considering biological sex. Generation of protective, neutralizing antibodies is a goal of vaccine development, with the cautionary note that in models of SARS-CoV vaccination some antibody responses induced potent inflammatory responses[57]. Persistence of antibodies, epitope targeting and non-neutralizing Fc-mediated antibody characteristics should be assessed with sex-stratified analyses. As vaccines are developed, the female bias towards both potent responses and adverse effects should be considered and sex-specific dosing should be tested, where appropriate[87].

Sex impacts the development of regulatory T cells[88–91], the distribution of lymphocyte subsets[92] and the overall quality of T cell responses[93,94]. In T cells, overexpression of X-encoded immune genes, including *CD40LG* and *CXCR3*, has been linked to incomplete X chromosome inactivation and T cell-specific epigenetic modifications of the X chromosome[95,96]. It is unknown whether T cell phenotypes contribute to COVID-19; data from the prior SARS outbreak did not link T cell responses to outcomes in humans[97], but mouse models suggest a role for $CD4^+$ T cells[98]. In patients with MERS, T cell responses were dysregulated[99], but sex differences were not analysed. In the current pandemic, lymphopenia is associated with severe disease[100,101], and early evidence suggests that the clinical markers of lymphocyte count may be lower in males and neutrophil–lymphocyte ratios may be higher[17]. Further work is needed to define the sex-differential role of T cells in acute infection, in acute lung injury phenotypes[102] and as potential vaccine targets.

Severe infection and acute respiratory distress syndrome. Severe cases of COVID-19 are typically marked by acute respiratory distress syndrome (ARDS), with respiratory failure requiring oxygen support and mechanical ventilation. The infection is primarily characterized by diffuse alveolar damage without a consistent pattern of cell infiltration[75,103–105]. The pathogenesis of ARDS involves the disruption of normal barrier function, inflammation and subsequent tissue repair. Whether there are sex-specific risks for ARDS and death from other causes, such as trauma, remains unknown[106,107], although there is a suggestion of a higher risk of lower respiratory tract infections among males[108] and that steroid hormones modulate the immune response to respiratory viral pathogens[109]. In one cohort of patients with COVID-19, severe respiratory failure was associated with a pattern of inflammation, macrophage activation and depletion of lymphocytes that was distinct from bacterial infection[110]. There was a sex bias for severe COVID-19 not observed in the comparator group with bacterial infections[110]. Sex-differential production of IL-6 (REF.[111]), monocyte transcriptional patterns and inflammatory set point[19,21,22] could contribute to an enhanced risk of death in males and highlight the importance of sex-stratified analyses to guide deployment of safe and effective immunomodulatory therapeutics for males and females[112].

Conclusions

Emerging data demonstrating more favourable outcomes for community-dwelling adult females across age strata offer an immediate opportunity for comparative biology experiments to define features of COVID-19 pathogenesis and the associated immune response. The research pipeline should integrate sex as a biological variable in all stages, from fundamental research to preclinical drug development, clinical trials and epidemiological analyses[113]. Considering the role of intersecting factors — including, but not limited to, gender, age, race and other identifying characteristics — is critical to understanding the biological and sociocultural factors contributing to heterogeneous COVID-19 outcomes. Sex is a driver of discovery and innovation[114], and taking a sex-informed approach to COVID-19 research[115] and medicine[116] will uncover novel features of the host immune response to SARS-CoV-2 and ultimately result in more equitable health outcomes.

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PLOS PATHOGENS

OPINION

Biological sex impacts COVID-19 outcomes

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Abstract

The current novel coronavirus disease 2019 (COVID-19) pandemic is revealing profound differences between men and women in disease outcomes worldwide. In the United States, there has been inconsistent reporting and analyses of male–female differences in COVID-19 cases, hospitalizations, and deaths. We seek to raise awareness about the male-biased severe outcomes from COVID-19, highlighting the mechanistic differences including in the expression and activity of angiotensin-converting enzyme 2 (ACE2) as well as in antiviral immunity. We also highlight how sex differences in comorbidities, which can be associated with both age and race, impact male-biased outcomes from COVID-19.

We are in the midst of a pandemic. Many of us predicted that the next "100 year pandemic" would be caused by an influenza A virus, like the H1N1 virus that caused the 1918 influenza pandemic. Instead, the current pandemic is caused by a novel β-coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Currently, there are almost 2 million cases and over 100,000 deaths worldwide from the disease caused by this virus, called the novel coronavirus disease 2019 (COVID-19). Like the 1918 influenza pandemic [1], men are at greater risk of more severe COVID-19 outcomes than women, with both sex (i.e., biological differences) and gender (i.e., sociocultural and behavioral differences) playing fundamental roles.

The initial reports from China, followed by data from several countries in Europe, have highlighted that there are roughly similar numbers of confirmed SARS-CoV2 cases between men and women. The severity of COVID-19, as measured by hospitalization, admission to intensive care units, and rates of fatality, however, has consistently been 2-fold greater for men than women [2], with the Global Health 50/50 research initiative providing real-time sex-disaggregated data from most countries worldwide [3]. Unfortunately, despite the United States currently having the most COVID-19 cases in the world, considerably less attention has been paid to sex-disaggregation of data than in Europe and China.

We took this opportunity to evaluate the current situation in the US to both determine if similar patterns of male–female differences are observed and to document which states are or

are not disaggregating and analyzing data by sex. As of this writing, 26 states have more than 2,000 confirmed cases (https://www.worldometers.info) and from these states, only three (Louisiana, New Jersey, and Pennsylvania) have not sex-disaggregated cases of COVID-19. New York has the greatest number of COVID-19 cases in the US and is an epicenter of this pandemic. Of the data from the remaining 23 states, 7 states replicate the epidemiological pattern seen in New York City (NYC) (**Fig 1**) and elsewhere in the world [3], in which numbers of COVID-19 cases are similar between men and women. The other 16 states, however, suggest a female-bias exists in COVID-19 cases (i.e., 1 to 0.9/0.8 male to female ratio). This includes Washington state, which is another epicenter of the COVID-19 pandemic in the US. Of 167 COVID-19 cases from a Washington state long-term care facility, a majority of cases were women (68% of residents and 76% of healthcare workers) [4]. The total number of men and women among facility residents and healthcare workers was not provided and with women living longer than men and being more likely to work as healthcare providers [5], gender-associated factors may be involved [5].

Of the 26 states analyzed, only two counties within two different states (i.e., NYC and Bucks County, Pennsylvania) have reported rates of hospitalization broken down by sex, and both report greater rates of hospitalization from COVID-19 among men than women (**Fig 1**). Lastly, of the 26 states with more than 2,000 confirmed COVID-19 cases, only 13 (New York, Michigan, California, Illinois, Texas, Washington, Connecticut, Indiana, Colorado, Ohio, North Carolina, Wisconsin, and Alabama) have disaggregated fatalities from COVID-19 by sex and consistently show that fatality rates are 2-fold greater for men than women (**Fig 1**). Gender-associated factors have been reviewed elsewhere [2, 5]; thus, we seek to focus on biological mechanisms that could impact male–female differences in severe COVID-19 outcomes to call attention to sex-associated factors that could potentially provide novel insights into therapeutic interventions.

Angiotensin-converting enzyme 2 (ACE2) is a monocarboxpeptidase that counteracts the vasoconstrictor effects of angiotensin (Ang)-(1–8) by converting this octapeptide hormone to the vasodilator heptapeptide Ang-(1–7) [6]. In 2003, ACE2 was found to be the SARS-CoV (i.e., the virus that caused the 2002 to 2003 SARS outbreak) receptor [7, 8], with SARS-CoV2

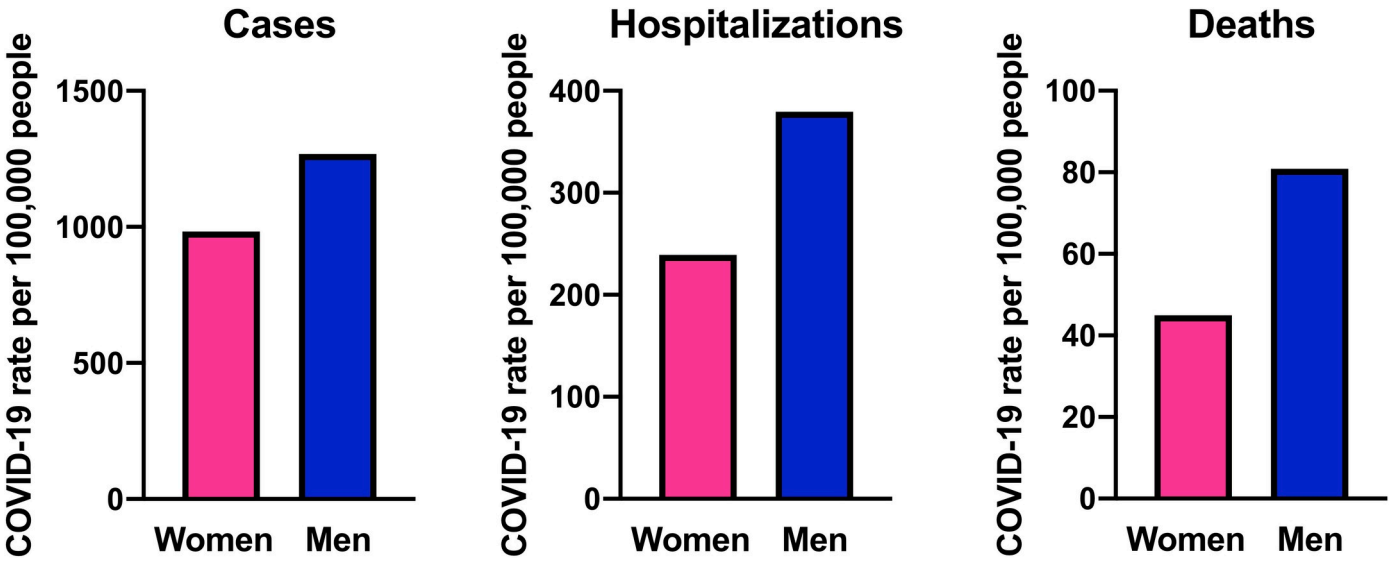

Fig 1. Sex-disaggregated numbers of COVID-19 cases, hospitalizations, and deaths per 100,000 people in NYC. Data were accessed from https://www1.nyc.gov/site/doh/covid/covid-19-data.page on April 11, 2020. COVID-19, novel coronavirus disease 2019; NYC, New York City.

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binding ACE2 with even higher affinity [9]. ACE2 is expressed primarily in the kidney, heart, and testes, with the highest expression occurring in the kidney; it is, however, also expressed in the lungs at much lower levels [10]. The SARS-CoV2 virus S1 spike protein binds to the ACE2 receptor in alveolar epithelial cells of the lungs. ACE2 protein is expressed in a sex-specific manner in the mouse kidney; male mice have nearly 2-fold higher levels of renal ACE2 protein than female mice [11]. Furthermore, using the four core genotype mouse model in which gonadal sex (ovaries versus testes) is separated from the sex chromosome complement (XX versus XY) [12], we found renal ACE2 activity was greater in the male kidney regardless of the sex chromosome complement [11]. This sex difference in renal ACE2 activity was driven by estradiol reducing ACE2 activity regardless of the sex chromosome complement. These findings have implications for the observed sex differences in COVID-19 outcomes. It will be important to study the sex-specific regulation of ACE2 in the lung and other tissues involved in COVID-19 pathogenesis including the heart and brain [6] and also to investigate whether estrogens protects women from COVID-19 by reducing the expression levels of the receptor for the SARS-CoV2 virus.

The innate recognition and response to viruses as well as downstream adaptive immune responses during viral infections also differ between females and males [13]. We and others have illustrated that females generally mount greater inflammatory, antiviral, and humoral immune responses than males during viral infections [14], which contributes to better clearance of viruses, including SARS-CoV [15]. Enhanced immunity in females can, however, also result in greater immunopathology and tissue damage at later stages of viral disease, such as during influenza A virus infection [16]. To date, we have only identified two COVID-19 studies that have disaggregated and analyzed immunological outcome data by sex. In a published study of 168 patients with severe COVID-19 in Wuhan, China, it was reported that men were significantly more likely to remain hospitalized and die and less likely to be discharged from the hospital during the study period than women [17]. The male–female difference was most pronounced among individuals 60 years of age and older. In this study, the neutrophil to lymphocyte ratio and serum C-reactive protein concentrations were twice as high in male as in female COVID-19 patients, as well as in patients who died compared with patients who were discharged from the hospital (not disaggregated by sex) [17]. These data suggest that inflammatory immune responses and cell counts might be more elevated in men and associated with worse outcomes from COVID-19 than in women.

Mounting evidence suggests that humoral immune responses can be measured not only to confirm exposure to SARS-CoV2 but also to assess adaptive immune responses necessary for clearance of SARS-CoV2. As a result, convalescent plasma transfer studies are underway for compassionate care of severe COVID-19 patients [18], without, however, consideration of the sex of the donor. In a not yet peer-reviewed study of 331 patients with confirmed SARS-CoV2 infections in Wuhan, China, anti–SARS-CoV2 immunoglobulin G (IgG) responses were measured and compared among patients with either clinically diagnosed mild or severe disease. The sex distribution of recovering cases was 36% and 65% for men and women, respectively [19]. Among patients with mild COVID-19, anti–SARS-CoV2 IgG titers were similar between the sexes. In contrast, among patients with severe disease, women exhibited greater antibody responses than men, with production of antibodies at earlier phases of disease suggesting one possible immunological mechanism mediating better recovery from COVID-19 in women than men [19].

Development of mouse models for SARS-CoV2 will be instrumental for mechanistically assessing the causes of sex differences in the pathogenesis of COVID-19. In a mouse model of SARS-CoV infection, female mice had lower virus titers and less severe pulmonary damage from monocyte–macrophage infiltration and cytokine production, resulting in lower mortality

in female (20%) compared with male (80%) mice [15]; a sex distribution similar to that observed in human SARS [20]. Notably, the endogenous production of estradiol in female mice was important for this protection.

Comorbidities that are associated with more severe outcomes from COVID-19 in the US, include diabetes, obesity, hypertension, heart disease, chronic kidney disease, and chronic pulmonary disease. Notably, diabetes, obesity, and hypertension are the top three conditions associated with fatal COVID-19 cases in China and Italy [4, 21–23]. As of April 14, 2020, in New York, hypertension accounted for 56.8% and diabetes 42.4% of fatal cases [24]. In Louisiana, where New Orleans is the epicenter by death rate per capita, hypertension accounted for 59.8%, diabetes 38.1% and obesity 22.3% of fatal cases [25]. To date, no study has reported whether these comorbidities are influenced by sex or gender in COVID-19 patients. Biological (sex) as well as behavioral (gender) factors contribute to differences between men and women in these comorbidities [26]; the sex and gender-associated factors that underlie these comorbidities, however, have not been evaluated in context of COVID-19. There also has been no consideration about how sex intersects with age and race to further increase risk of severe COVID-19 outcomes in men, despite observations illustrating that older aged individuals [27] and African Americans [28] are also at risk for severe COVID-19 outcomes. For these reasons, we call on clinicians and epidemiologists to report data pertaining to comorbidities associated with COVID-19 disaggregated by sex, age, and race. We also emphasize the importance of considering the biological variable of sex when conducting basic science studies of COVID-19.

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