MAYO CLINIC

MAYO CLINIC PROCEEDINGS

ORIGINAL ARTICLE

Artificial Intelligence-Augmented Electrocardiogram in Determining Sex: Correlation with Sex Hormone Levels

Jwan A. Naser, MBBS; Francisco Lopez-Jimenez, MD, MSc, MBA; Alice Y. Chang, MD; Abraham Baez-Suarez, PhD; Zachi I. Attia, PhD; Sorin V. Pislaru, MD, PhD; Patricia A. Pellikka, MD; Grace Lin, MD, MBA; Suraj Kapa, MD; Paul A. Friedman, MD; and Peter A. Noseworthy, MD

Abstract

Objective: To study the relationship between the sex probability derived from the artificial intelligence (AI)-augmented electrocardiogram (ECG) and sex hormone levels.

Patients and Methods: Adult patients with total testosterone (TT; ng/dL) or estradiol (E2; pg/mL) levels (January 1, 2000, to December 31, 2020) with ECGs obtained within 6 months of the blood sample were identified. The closest ECG to the blood test was used. The AI-ECG model output ranges from 0.0 to 1.0, with higher numbers indicating high probability of being male. Low male probability was defined as ≤0.3, intermediate as 0.31 to 0.69, and high as ≥0.7. Continuous variables are expressed as median (interquartile range).

Results: Paired TT-ECGs were available in 58,084 male subjects and 11,190 female subjects. Paired E2-ECGs were available in 2835 male patients and 18,228 female patients. TT levels had moderate positive correlation with AI-ECG male sex probability (r=0.46, P<.001). Male subjects with low AI-ECG male sex probability had lower TT and higher E2 levels compared with men with high probability (TT: 303 [129-474] vs 381 [264-523], P <.001; E2: 35 [21-49] vs 32 [22-38], P=.05). Female subjects with high AI-ECG male sex probability had higher TT and lower E2 levels compared with those who had low male probability (TT: ≤50 years of age: 31 [18-55] vs 26 [16-39], P<.001; >50 years of age: 27 [12-68] vs 20 [12-34], P<.001; E2: ≤50 years of age: 58 [30-124] vs 47 [25-87], P=.001; >50 years of age: 30 [10-55] vs 21 [10-41], P=.006).

Conclusion: In this study, TT levels were lower and E2 levels higher with decreasing AI-ECG male probability in both sexes. Male and female patients with discordant AI-ECG sex probability had significantly different TT or E2 levels. This suggests that the ECG could be used as a biomarker of hormone status.

 ■ Mayo Clin Proc. 2023;98(4):541-548

The impact of sex on the electrical activity of the heart has long been known.[1,2] Differences in electrocardiograms (ECGs) between the 2 sexes have been well described.[1-3] Some of these differences have rendered sex an independent risk factor for different types of arrhythmias[2,4]; for example, men are at increased risk of atrial fibrillation and Brugada syndrome, whereas women have higher risk of Torsades de pointes.[5-7] Furthermore, it was noted in multiple studies that manipulation of sex hormone levels resulted in directly observed changes in ECGs.[1,7-9]

Recently, ECGs enhanced with artificial intelligence (AI) have demonstrated the ability to identify information not readily detectable by the human eye.[10-12] The AI-enabled 12-lead ECG using a convolutional neural network was able to identify the self-reported sex in a population undergoing clinically indicated ECGs with an area under curve (AUC) of 0.97 and accuracy of 90.4%.[12] In this model, the AI-ECG gives a

From the Department of Internal Medicine (J.A.N.); Department of Cardiovascular Medicine (J.A.N., F.L.-J., A.B.-S., Z.I.A., S.V.P., P.A.P., G.L., S.K., P.A.F., P.N.); and Department of Endocrinology and Metabolism (A.Y.C.), Mayo Clinic, Rochester, MN.

probability number of being male between 0.0 and 1.0, with higher numbers indicating high probability of being male and lower numbers indicating high probability of being female.[12] However, given that sex hormone levels were not assessed, the correlation of the AI-enabled ECG sex probability with testosterone and estrogen levels is unknown. Moreover, despite the high accuracy of the model, there was a small fraction of patients who had discordance between the AI-ECG sex probability and their true sex.[12] Whether this discordance was caused by actual differences in sex hormone levels in this small fraction of patients remains to be investigated.

In this study, we sought to assess the correlation between the AI-ECG-derived sex probability and sex hormone levels—specifically, total testosterone (TT) and estradiol (E2) levels—and to compare sex hormone levels in patients with a discordant AI-ECG sex probability with those with a sex probability concordant with their true sex.

METHODS

Study Population

The study was approved by the Institutional Review Board (IRB). Only patients who had previously agreed to include their data in a retrospective chart review research study were included. Consecutive adult patients in whom TT (measured in ng/dL) or E2 (measured in pg/mL) levels were obtained between 2000 and 2020 and had an ECG obtained within 6 months of the blood sample were identified from an institutional database. Because of the retrospective nature of the study and the inability to exclude interventions with impact on sex hormone levels in the 6 months surrounding the blood sample, patients who had multiple ECGs within 6 months of the blood sample and had differences exceeding an arbitrary cutoff value of 0.25 in the AI-ECG probability of male sex were excluded. Baseline demographic data, comorbidities, laboratory data, and ECGs were extracted from the electronic medical records. Sex hormone levels, including TT and E2, were extracted from the electronic medical records.

Artificial Intelligence-Enabled ECGs

Data from the AI-enabled ECGs was extracted using the previously trained AI platforms.[12] No additional training was done for the purpose of this study. When multiple ECGs were available within the 6-month period around the time of the sex hormone levels, the closest ECG to the blood sample was paired with TT or E2 levels; TT and E2 datasets were analyzed separately. In patients who had more than 1 paired ECG-blood test, the first chronologic pair was used. This way, only 1 ECG and 1 blood sample from each patient were analyzed. Low male probability was defined as $\leq$0.3, intermediate as 0.31 to 0.69, and high as $\geq$0.7. These terms are interchangeable with high, intermediate, and low female probability, respectively. The used cut points are based on the original study of the involved AI platform,[12] which showed improved accuracy of the algorithm from 90.4% to 94.3% when the 10% ECGs with intermediate male sex probability (0.31< probability <0.69) were excluded.

Statistical Analysis

Continuous variables are expressed as median and interquartile range (IQR). Categorical variables are expressed as frequency and percentage. Normal distribution was assessed with the Shapiro-Wilk statistic. Normally distributed continuous variables were compared using the Student's *t*-test, and non-normal variables with the Wilcoxon test. Multiple comparison tests were used when comparing more than 2 entities. Correlations among variables were evaluated using the Spearman's test. Female subjects were analyzed as 2 groups (likely premenopausal vs likely postmenopausal) according to an age cutoff of >50 years (or $\geq$51 years). Statistical significance was considered for *P* value <0.05. All analyses were performed with JMP Pro software version 14.1.0 (SAS Institute Inc).

Mayo Clin Proc. ■ April 2023;98(4):541-548 ■ https://doi.org/10.1016/j.mayocp.2022.08.019
www.mayoclinicproceedings.org

RESULTS

Total Testosterone Level and the AI-ECG Sex Probability

A total of 79,695 patients with paired TT-ECG data were identified, of whom 10,421 were excluded based on a difference in the AI-ECG male sex probability of more than 0.25, as detailed in the Methods section. Overall, 69,274 patients (median age 59 [49-69] years) comprised the TT study cohort. ECGs were obtained within median 3 (IQR: 1-33) days of the TT blood test. There was a moderate positive correlation between TT levels and the AI-ECG male sex probability (r=0.46, P<.001). Upon stratifying according to age, this correlation was stronger in younger patients ≤50 years of age (r=0.60, P<.001) than in patients >50 years of age (r=0.36, P<.001), (Table 1; Supplemental Figure, available online at http://www.mayoclinicproceedings.org). Results remained the same when excluding patients with ECGs included in the training of the original algorithm (3713 of 79,695 patients) with r=0.45 overall, r=0.59 for patients ≤50 years of age, and r=0.36 for patients >50 years of age (P<.05 for all).

There were 58,084 male patients with paired TT-ECG data (median age 61 [52-70] years). Figure 1 shows proportions in each of the AI-ECG male sex probability categories (high, intermediate, and low). On the other hand, there were 11,190 female patients with TT data (median age 47 [35-58] years) (Figure 1). In female patients ≤50 years of age, 227 (4%) had high AI-ECG male sex probability, 469 (7%) had intermediate AI-ECG male sex probability, and 5751 (89%) had low AI-ECG male sex probability. These numbers were 252 (5%), 437 (9%), and 4052 (85%), respectively, for female patients >50 years of age.

TT data in the 3 categories of AI-ECG sex probability in male patients, female patients ≤50 years of age, and female patients >50 years of age are shown in Figure 2. Male subjects with discordantly low AI-ECG male sex probability had lower TT levels compared with male patients with concordantly high AI-ECG male sex probability (303 [129-474] vs 381 [264-523] ng/dL, P<.001]. Female patients with discordantly high AI-ECG male sex probability had higher TT levels compared with those with concordantly low male probability (≤50 years of age: 31 [18-55] vs 26 [16-39] ng/dL, P<.001; >50 years of age: 27 [12-68] vs 20 [12-34] ng/dL, P<.001). Results were similar when excluding patients with ECGs included in the training of the original algorithm.

TABLE. The Correlation Between Total Testosterone Levels and the AI-ECG Male Sex Probability

	Correlation coefficient (r)	Coefficient of determination (r^2)
All patients	0.46	0.21
Patients aged ≤ 50 years	0.60	0.36
Patients aged > 50 years	0.36	0.13

AI-ECG = artificial intelligence-augmented electrocardiogram.

Estradiol Level and the AI-ECG Sex Probability

A total of 22,792 patients with E2 levels were identified. Of those, 1729 were excluded, and 21,063 patients (median age 48 [40-55] years of age) were analyzed. ECGs were obtained within median 4 (IQR: 0-54) days of the E2 level. There was only a weak negative correlation between AI-ECG male sex probability and E2 levels (r=−0.1, P<.001).

There were 2835 male patients (median age 58 [46-68] years of age) with paired E2-ECG levels. Figure 1 shows proportions in each of the AI-ECG male sex probability categories. There were 18,228 female patients (median age 48 [39-54] years of age) with paired ECG-E2 levels (Figure 1). There were 10,545 female patients ≤50 years old (304 [3%] had high AI-ECG male sex probability; 653 [6%] had intermediate AI-ECG male sex probability; 9588 [91%] had low AI-ECG male sex probability) and 7683 female patients aged >50 years (300 [4%] had high AI-ECG male sex probability;

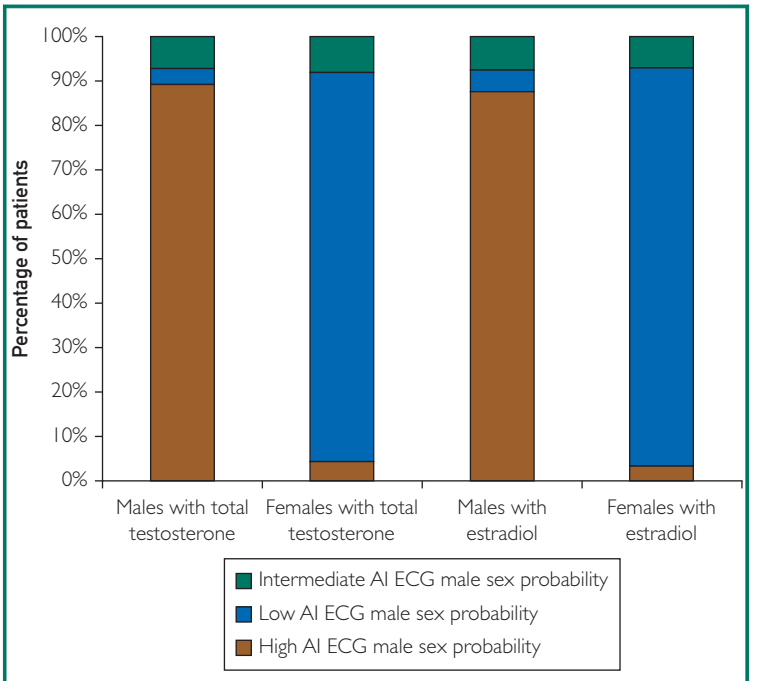

FIGURE 1. Distribution of high, intermediate, and low artificial intelligence-augmented electrogardiogram (AI-ECG) male sex probability in men and women with total testosterone and estradiol levels. Note that ≥88% of men and women had concordant AI-ECG sex probability (ie, men with high AI-ECG male sex probability [brown] or women with low AI-ECG male sex probability [blue]). Also, ≤5% had discordant probability (ie, men with low AI-ECG male sex probability [blue] or women with high AI-ECG male sex probability [brown]). Seven to eight percent of men and women had intermediate AI-ECG sex probability (green).

618 [8%] had intermediate AI-ECG male sex probability; 6765 [88%] had low AI-ECG male sex probability).

E2 data in the 3 categories of AI-ECG sex probability in male patients, female patients ≤50 years of age, and female patients >50 years of age are shown in Figure 3. Male patients with discordantly low AI-ECG male sex probability had higher E2 levels compared with those who had concordantly high male probability (35 [21-49] vs 32 [22-38] pg/mL, P=.05). Female patients with discordantly high AI-ECG male sex probability also had lower E2 levels compared with those who had concordantly low male probability (≤50 years of age: 47 [25-87] vs 58 [30-124] pg/mL, P=.001; >50 years of age: 21 [10-41] vs 30 [10-55] pg/mL, P=.006].

Results were similar when excluding patients with ECGs included in the training of the original algorithm (1356 of 22,792 patients).

DISCUSSION

The current study investigated the correlation between the sex probability derived from the previously trained AI-augmented ECG platform and sex hormone levels.[13] Our major findings were that the AI-augmented ECG male sex probability correlated well with TT levels, especially in adults ≤50 years of age; the AI-ECG male probability had a dose-response relationship with TT and E2 levels in both sexes; and male and female patients with discordant AI-ECG sex probability had significantly different TT or E2 levels from those with concordant sex probability.

Sex hormones are known to interact with nuclear and membrane receptors of the cardiac myocytes.[1,7,9] This interaction modulates the function of the ion channels that regulate the electrical activity of the heart.[1,7,9] Some of the resulting manifestations in the ECG include changes in the heart rate, PQ interval, QT interval, and J point height, among others.[7-9,13] The AI-augmented ECG was shown to be able to identify the signature of male and female sex with an AUC of 0.97 and accuracy of 90.4%, further emphasizing the interaction between sex and ECG signals.[12] Because a convolutional neural network was used to train the algorithm using 12-lead ECG recordings, the precise ECG parameters used to differentiate patients' sex in this AI-ECG tool are unknown. Nonetheless, in this study, we demonstrated that TT levels correlated well with the male sex probability derived from the AI-enabled ECG. This correlation was noted to be stronger in younger patients with a correlation coefficient of 0.60, which decreased significantly in older patients (correlation coefficient of 0.36). The decreased correlation of the AI-ECG male sex probability with TT level in older adults may indicate that testosterone level, which is known to decline with aging,[14] becomes less important in affecting the ECG in

Mayo Clin Proc. ■ April 2023;98(4):541-548 ■ https://doi.org/10.1016/j.mayocp.2022.08.019
www.mayoclinicproceedings.org

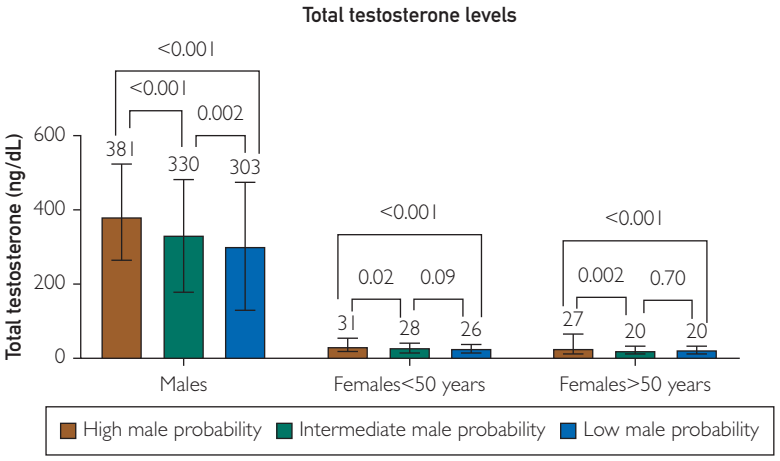

FIGURE 2. Total testosterone levels across the 3 categories (high, intermediate, low) of artificial intelligence-augmented electrocardiogram (AI-ECG) male sex probability in men and women. Men with discordantly low AI-ECG male sex probability had significantly lower total testosterone levels compared with men with higher AI-ECG male sex probability. On the other hand, women with discordantly high AI-ECG male sex probability had significantly higher total testosterone levels compared with women with low AI-ECG male sex probability. Values typed above bars represent the median. *P* values comparing bar values are shown.

the elderly, and other undefined factors that are probably less sex specific may be involved. This could also be implicated in the decreased accuracy of the AI-ECG sex probability in older adults (89% compared with 93% in younger adults).[13]

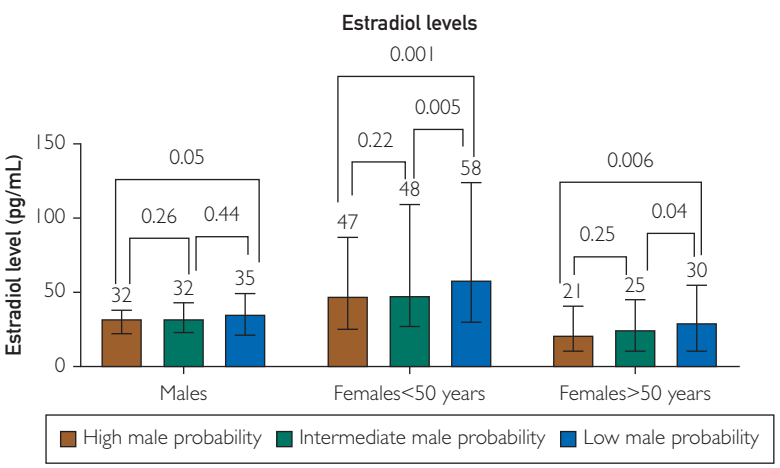

FIGURE 3. Estradiol levels across the 3 categories (high, intermediate, low) of artificial intelligence-augmented electrocardiogram (AI-ECG) male sex probability in men and women. Men with discordantly low AI-ECG male sex probability had higher estradiol levels with borderline association compared with men with high AI-ECG male sex probability. On the other hand, women with discordantly high AI-ECG male sex probability had significantly lower total testosterone levels compared with women with low AI-ECG male sex probability. Values typed above bars represent the median. *P* values comparing bar values are shown.

Despite the high accuracy of the AI-ECG sex probability algorithm, a fraction of patients had discordance between the AI-ECG sex probability and their true sex (ie, male patients with low AI-ECG sex probability of being male or female with high AI-ECG sex probability of being male).[12] In this study, we found that ≤5% of men with TT or E2 levels had discordant AI-ECG sex probabilities. These men were noted to have significantly lower TT levels and potentially higher—with borderline association—E2 levels compared with men with concordant AI-ECG sex probability who comprised ≥89% of male patients included. Similarly, ≤5% of female patients with TT or E2 levels had discordant AI-ECG sex probability and had significantly higher TT and significantly lower E2 levels compared with female patients with concordant AI-ECG sex probability, representing ≥85% of the female patients included. The intermediate range of the AI-ECG sex probability of being male (0.3-0.7) was observed in 7% to 8% of men and 6% to 9% of women. Male patients in the intermediate group had TT levels in the middle range between the levels observed in those with high and low male probabilities. In women, the intermediate group had comparable TT levels but significantly lower E2 levels than women with concordant AI-ECG probability and had significantly lower TT levels but similar E2 levels to female patients with discordant AI-ECG sex probability. These findings indicate that the discordance observed between the true sex and the AI-ECG sex probability is likely influenced to some extent by sex hormone levels.

We also noted that TT level was more important than E2 level in men, although both were important in women. Moreover, E2 levels had a much weaker correlation with the AI-ECG–derived male sex probability compared with TT level. This is consistent with a study by Fülöp et al,[9] which demonstrated that manipulating testosterone levels in male and female dogs affected QT and QTc intervals and QTc-dispersion, whereas manipulating estrogen levels did not alter parameters other than QTc-dispersion. Fülöp et al further showed that the repolarization of canine ventricular myocardium was affected by testosterone—but not estrogen—levels in both sexes.[9] Zhang et al also demonstrated that testosterone levels were implicated in cardiac repolarization and determining QT interval in humans in a multicenter cohort study with only a marginal effect of estrogen levels observed solely in the subgroup including postmenopausal women.[15]

The current study suggests that the fingerprint signal detected by the ECG is hormone mediated. The correlation between the AI-ECG sex probability and sex hormone levels may have important clinical implications (eg, a man with low AI-ECG male sex probability is likely to have a lower testosterone level; this could facilitate screening for symptoms of testosterone deficiency, such as decreased libido and checking testosterone levels, if appropriate). Moreover, the longitudinal role of the AI-ECG sex probability may be promising. In a previous study, we reported the effect of Graves disease on the AI-ECG in women and found that the AI-ECG sex probability of being female decreased with the diagnosis of Graves disease and increased again after restoration of euthyroidism.[16] In that study, we also noted that women <45 years of age who had menstrual changes as part of their presenting symptoms of thyrotoxicosis had lower AI-ECG female sex probability compared with women with no menstrual changes.[16] If the correlation between the AI-ECG sex probability tool and sex hormone levels were validated in future longitudinal prospective studies, this inexpensive tool could be used as a biomarker to provide additional information on sex hormone levels when ECGs are done for cardiac indications without directly obtaining sex hormone levels. This could involve monitoring androgen levels in men with prostate cancer on androgen deprivation therapy, following response to hormone replacement therapy in transgender persons and in patients with hypogonadism, tracking the transition through menopause in women and potentially identifying diseases that involve changes in sex

Mayo Clin Proc. ■ April 2023;98(4):541-548 ■ https://doi.org/10.1016/j.mayocp.2022.08.019
www.mayoclinicproceedings.org

hormone levels, such as premature ovarian failure.

Limitations

Our study had several limitations. First, this was a retrospective study in patients who had a clinical indication for assessment of TT or E2 levels. Therefore, the correlation between the AI-ECG sex probability and sex hormone levels in the general healthy population cannot be inferred from the current study. For the same reason, it was not possible to exclude interventions with a potential impact on sex hormones levels between the time of the blood test and the time of the ECG. However, patients who had differences in the AI-ECG—derived sex probability exceeding 0.25 in the ECGs obtained within 6 months of the blood sample were excluded to minimize such potential effect. We also were not able to ascertain what the specific indication for sex hormone evaluation was, including why some patients had only TT, only E2, or both TT and E2 levels obtained. The cut point of >50 years was used as an estimate for menopausal hormone level changes. Finally, the effects of cyclic variations of hormone levels could not be controlled in this retrospective study and could have affected the study results. Prospective studies in controlled settings are needed to further validate the AI-ECG tool in determining sex probability and its potential impact on screening for hormonal imbalances.

CONCLUSION

The AI-ECG-derived sex probability correlated well with TT levels, especially in patients ≤50 years of age, and had a dose-response relationship with TT and E2 levels in both sexes. Moreover, male and female patients with discordant AI-ECG sex probability had significantly different TT or E2 levels from those with concordant sex probability. Our findings indicate that sex hormones affect the ECG in ways that can be identified by a convolutional neural network and suggest that the ECG could be used as a biomarker of hormone status.

POTENTIAL COMPETING INTERESTS

The authors report no competing interests.

ACKNOWLEDGMENTS

All authors have participated in each of the following: conception and design, acquisition of data, or analysis and interpretation of data; drafting the manuscript or revising it critically for important intellectual content; and final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **AI,** artificial intelligence; **AUC,** area under curve; **E2,** estradiol; **ECG,** electrocardiogram; **IQR,** interquartile range; **TT,** total testosterone

Correspondence: Address to Peter A. Noseworthy, MD, Division of Cardiovascular Diseases, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905 (Noseworthy.Peter@mayo.edu; Twitter: @noseworthypeter).

REFERENCES

1. Costa S, Saguner AM, Gasperetti A, Akdis D, Brunckhorst C, Duru F. The link between sex hormones and susceptibility to cardiac arrhythmias: from molecular basis to clinical implications. *Front Cardiovasc Med*. 2021;8:644279.
2. Salama G, Bett GC. Sex differences in the mechanisms underlying long QT syndrome. *Am J Physiol Heart Circ Physiol*. 2014;307(5):H640-H648.
3. Bazett HC. An analysis of the time-relations of electrocardiograms. *Heart*. 1920;7:353-370.
4. Kurokawa J, Kodama M, Clancy CE, Furukawa T. Sex hormonal regulation of cardiac ion channels in drug-induced QT syndromes. *Pharmacol Ther*. 2016;168:23-28.
5. Meyer S, van der Meer P, van Tintelen JP, van den Berg MP. Sex differences in cardiomyopathies. *Eur J Heart Fail*. 2014;16(3):238-247.
6. Di Diego JM, Cordeiro JM, Goodrow RJ, et al. Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males. *Circulation*. 2002;106(15):2004-2011.
7. Magnani JW, Moser CB, Murabito JM, et al. Association of sex hormones, aging, and atrial fibrillation in men: the Framingham Heart Study. *Circ Arrhythm Electrophysiol*. 2014;7(2):307-312.
8. van Noord C, Dörr M, Sturkenboom MC, et al. The association of serum testosterone levels and ventricular repolarization. *Eur J Epidemiol*. 2010;25(1):21-28.
9. Fülöp L, Bányász T, Szabó G, et al. Effects of sex hormones on ECG parameters and expression of cardiac ion channels in dogs. *Acta Physiol (Oxf)*. 2006;188(3-4):163-171.
10. Attia ZI, Noseworthy PA, Lopez-Jimenez F, et al. An artificial intelligence-enabled ECG algorithm for the identification of

patients with atrial fibrillation during sinus rhythm: a retrospective analysis of outcome prediction. *Lancet*. 2019; 394(10201):861-867.

11. Attia ZI, Kapa S, Lopez-Jimenez F, et al. Screening for cardiac contractile dysfunction using an artificial intelligence-enabled electrocardiogram. *Nat Med*. 2019;25(1):70-74.
12. Attia ZI, Friedman PA, Noseworthy PA, et al. Age and sex estimation using artificial intelligence from standard 12-lead ECGs. *Circ Arrhythm Electrophysiol*. 2019;12(9):e007284.
13. Malik M, Hnatkova K, Kowalski D, Keirns JJ, van Gelderen EM. QT/RR curvatures in healthy subjects: sex differences and covariates. *Am J Physiol Heart Circ Physiol*. 2013;305(12):H1798-H1806.
14. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. *J Clin Endocrinol Metab*. 2001;86(2):724-731.
15. Zhang Y, Ouyang P, Post WS, et al. Sex-steroid hormones and electrocardiographic QT-interval duration: findings from the third National Health and Nutrition Examination Survey and the Multi-Ethnic Study of Atherosclerosis. *Am J Epidemiol*. 2011;174(4):403-411.
16. Naser JA, Attia Z, Pislaru S, et al. Effect of Graves' disease on female probability derived from artificial intelligence-enabled electrocardiograms in women. *Circulation*. 2021;144(Suppl 1). A9974-A9974 (abstract).