Third Issue / December 2021

GENOMIC

In this issue

About Us

Genomic Prediction provides advanced genomic tests which improve IVF health outcomes. We offer IVF patients a cost-effective means to evaluate genetic risk due to chromosomal abnormality (ploidy), single-gene mutations, structural rearrangements, and polygenic diseases.

In May 2018, Genomic Prediction Clinical Laboratory was incorporated. Clinical Laboratory work upon human embryos began, initiating a year of extensive validation before the first clinical use.

In May 2019, Genomic Prediction published in European Journal of Human Genetics its first, formal validation of its flagship testing product, Expanded Preimplantation Genetic Testing. Shortly after this validation, the method was applied to the first case in history of PGT-P - reducing risk of breast cancer in a family with a family history of the disease, but no monogenic targets like BRCA1 or BRCA2. Months later, the first pregnancy with the method was announced.

As of May 2020, Genomic Prediction is a prestigious genetic testing company with global reach, a client map boasting partnerships and clinics on every continent, a track record of hundreds of completed clinical cycles and pregnancies, and a publication record of extensive validation in the most prestigious scientific journals in the world. Genomic Prediction has invented, validated, and implemented the first genetic miscarriage predictor in embryos, preimplantation genetic testing for polygenic disorders in humans, and the worlds first genomic index for risk ranking. These innovations to IVF adorn the world's most advanced PGT-A, PGT-M, and PGT-SR platform.

Genomic Prediction has fundamentally improved upon existing methods, and offers IVF parents the next step in embryo genetic testing, suitable for the information age.

By the numbers:

241 LifeView Provider Clinics and Laboratories

309 Publications in Peer Reviewed Science Journals

26 Countries Offering LifeView

5 Registered, Ongoing Clinical Trials

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PGT-A Capabilities: LifeView vs NGS

Genomic Prediction Clinical Laboratory

	NGS	LifeView™
Detects standard trisomy, monosomy	✓	✓
Superior detection accuracy combination of copy number, genotyping, and uniform coverage means higher accuracy than NGS	✗	✓
Detects segmental aneuploidy	✓	✓*
Determines origin of aneuploidy, via genotyping	✗	✓**
Performs fingerprinting: confirms and quantifies relatedness of any sample pair, via genotyping	✗	✓
Detects contamination, via genotyping	✗	✓
Detects haploidy and all forms of triploidy, via genotyping	✗	✓
Detects uniparental disomy, via genotyping	✗	✓
Can upgrade to include PGT-P embryo ranking, same biopsy	✗	✓
Can upgrade to include M2 miscarriage risk, same biopsy	✗	✓

* >800K probes, highest available resolution in the industry

** Parental saliva required

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LifeView™
Choice is better than chance

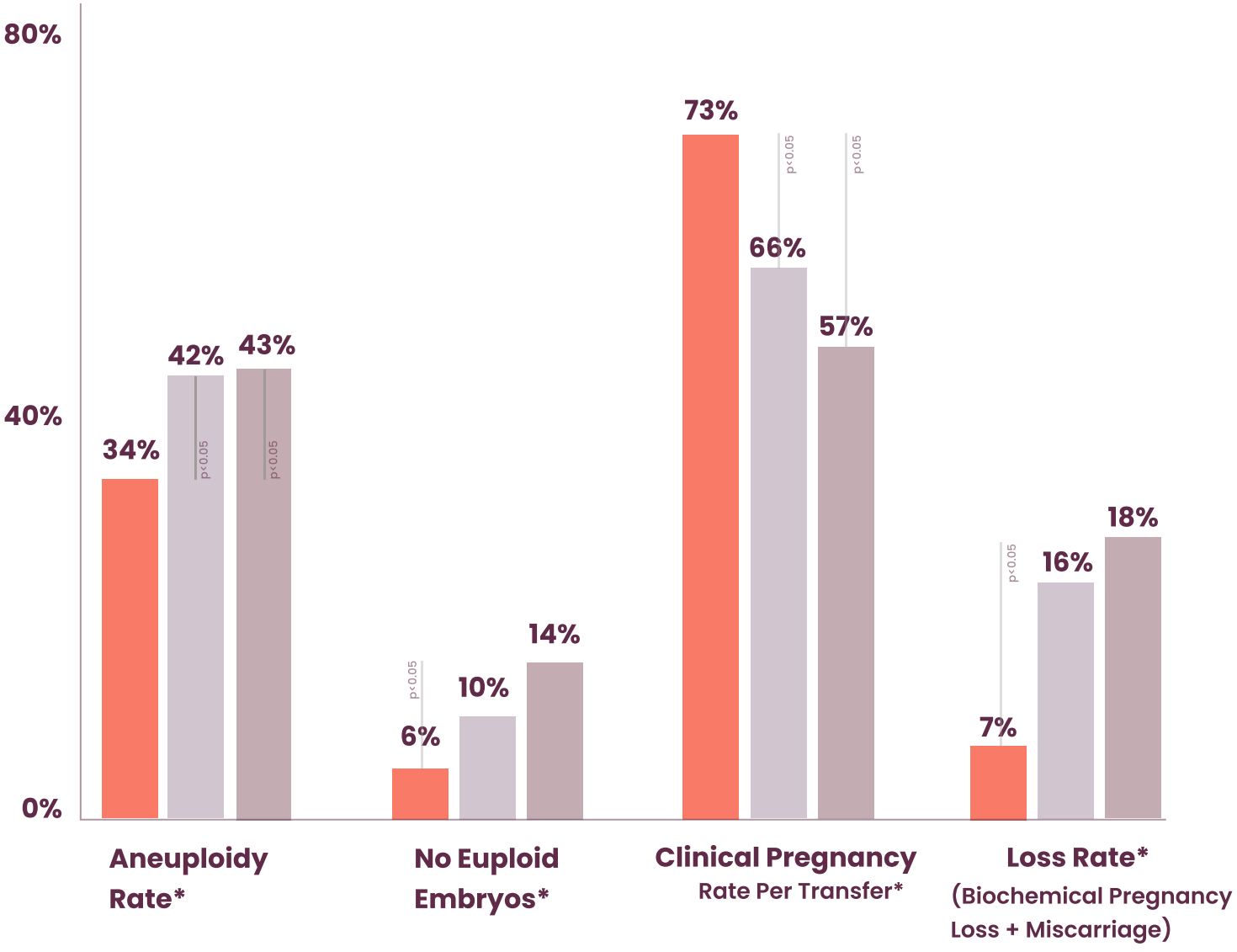
Better Outcomes

LifeView vs NGS

Same clinic
Same time period
Matched patient demographics

LifeView (GPCL)
NGS (PGT Reference Lab 1)
NGS (PGT Reference Lab 2)

n=3,278



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PGT-A
Testing for Aneuploidy

Genomic Prediction
Clinical Laboratory

Testing for aneuploidy (PGT-A)

Preimplantation Genetic Testing for Aneuploidy (PGT-A) is a genetic test performed on an embryo biopsy, to determine whether the embryo has the usual chromosome count. "Euploidy" refers to an embryo with the usual chromosome count. "Aneuploidy" refers to an embryo that does not have the usual count. Aneuploidy is the most common genetic cause of fertility problems, such as implantation failure and miscarriage. PGT-A tests for it.

How common is embryo aneuploidy?

All would-be parents have a risk of aneuploidy. This risk increases with maternal age:

Age	Risk	Age	Risk
30	23.2%	38	47.9%
31	31%	39	52.9%
32	31.1%	40	58.2%
33	31%	41	68.9%
34	31.3%	42	75.1%
35	34.5%	43	83.4%
36	35.5%	44	88.2%
37	42.6%	45	84.3%

PMID: 24355045

What is the benefit of PGT-A?

PGT-A will help you and your doctor decide which embryos to prioritize for transfer. PGT-A:

- Decreases the risk of implantation failure
- Decreases the risk of miscarriage
- Decreases the risk of certain health problems in an ongoing pregnancy
- Decreases the risk of multiple pregnancy, improving the success rate of single embryo transfer (SET)
- Decreases the time to conceive

What is LifeView™ PGT-A able to detect?

LifeViewTM PGT-A tests whether embryos have a whole extra chromosome, called a "trisomy", or a missing chromosome, called a "monosomy". It also tests for added or missing pieces of chromosomes, called "segmental aneuploidy". LifeView™ advanced testing also detects issues such as polyploidy, and uniparental disomy. LifeView™ PGT-A screens for a broad range of chromosome issues, increasing your chance of a healthy pregnancy.

What makes LifeView™ a better choice?

LifeView™ ensures the quality of your results:

Fingerprinting: LifeView™ checks whether the embryo being tested is genetically related to the other embryos in the same cycle, reducing the risk of sample mixups due to human error.

Contamination check: LifeView™ will check the embryo biopsy for contamination by other DNA. This check reduces the risk of misdiagnosis.

Superior accuracy: LifeView™ PGT-A combines copy number, genotyping and uniform coverage in a way that is improved from older NGS technology, offering comprehensive results with superior resolution and accuracy.

Can LifeView™ PGT-A be expanded?

LifeView™ offers more choice, without the need for additional embryo biopsy samples.

PGT-P: LifeView™ is the only platform validated to accurately predict each embryos lifetime risk for polygenic disorders; diseases influenced by variants in many genes, such as diabetes and certain cancers. Deciding which embryos to prioritize for transfer using LifeView™ PGT-P has been validated to reduce incidence of these disorders later in life.

PGT-SR: LifeView™ for structural rearrangements identifies extra or missing chromosomal material related to parental chromosome rearrangement.

PGT-M: LifeView™ for monogenic disorders is designed for individuals or couples at high risk of passing on a monogenic, or single gene, disorder.

M2: M2 is a genetic variant associated with increased risk of pregnancy complications and miscarriage. For couples with M2, LifeView™ can can be expanded to include M2 haplotype testing, to help you and your doctor make informed interventions to reduce the risk of miscarriage.

Who can I ask questions about LifeView™?

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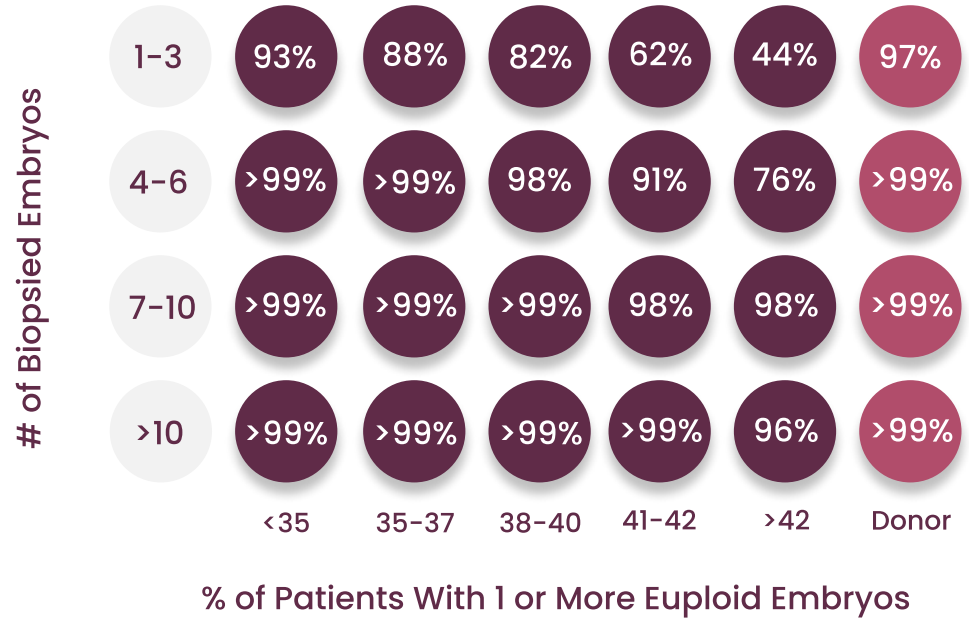
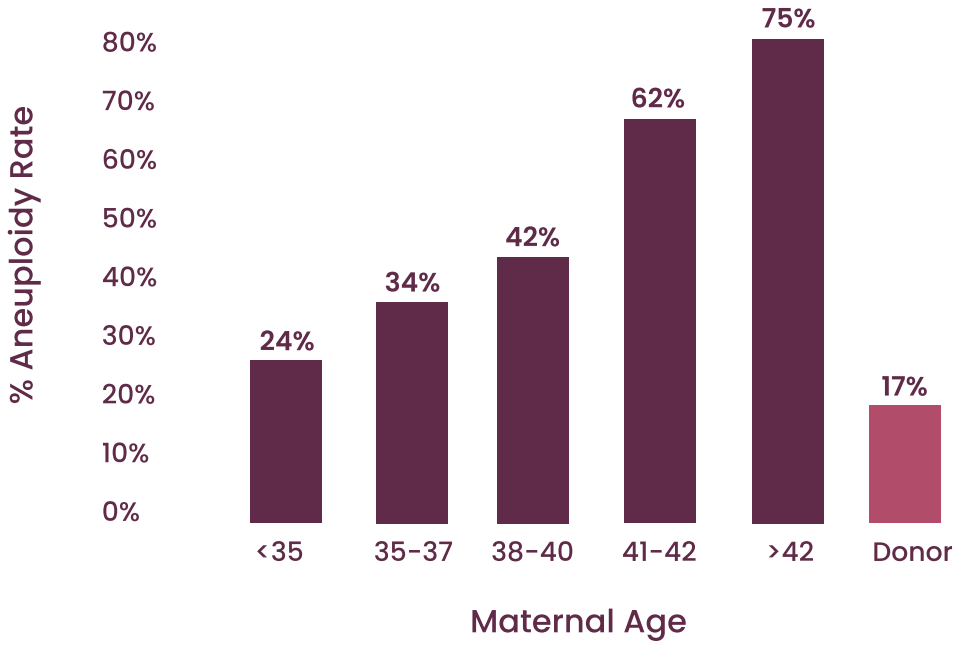
LifeView™ PGT-A

Genomic Prediction
Clinical Laboratory





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Genomic Prediction Clinical Laboratory on Mosaicism in PGT-A

Genomic Prediction Clinical Laboratory

Mosaicism in the context of PGT-A refers to the presence of both normal (euploid) and abnormal (aneuploid) cells in the embryo. Some PGT-A laboratories claim to be capable of detecting mosaicism. However, the clinical predictive value of these methods has proven to be 0% (1).

This is due to methodological inaccuracy that leads to significant false positives. Simple thresholds (i.e. 20-80%) overestimate the prevalence of mosaicism and lead to inappropriate classification. At least one third of embryos designated as "mosaic" are actually uniformly euploid, and another one third are uniformly aneuploid (2).

This explains both the observed reduction in success rates of embryos designated as "mosaic" (actually uniformly aneuploid), as well as the observed success of embryos designated as "mosaic" (actually uniformly euploid). To avoid misclassification of embryos as mosaic, Genomic Prediction Clinical Laboratory has developed a more rigorous method (LifeView PGT-A) that incorporates multiple types of data (not simple thresholds) and more accurately classifies embryos as euploid or aneuploid only (3).

This has led to a significant increase in the number of embryos available for transfer, significant increase in clinical pregnancy rates, and a significant reduction in miscarriage rates compared to laboratories reporting mosaicism (4).

References

1. Treff NR and Marin D. The "Mosaic" Embryo: Misconceptions and Misinterpretations in PGT-A. Fertility and Sterility. 2021. In Press
2. Marin D, Xu J, and Treff NR. Preimplantation genetic testing for aneuploidy: A review of published blastocyst reanalysis concordance data. Prenatal Diagnosis. 2020. 41(5):545-553
3. Treff et al. Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform. European Journal of Medical Genetics. 2019. 62(8):103647
4. http://online.anyflip.com/rough/dkyg/mobile/index.html

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Genomic Prediction Clinical Laboratory Guide to Ordering Embryo Health Scores (PGT-P)

Genomic Prediction Clinical Laboratory

Patients using any LifeView PGT service have the option to upgrade testing to include a more advanced screening result called an Embryo Health Score (EHS), which determines the combined risk of several common polygenic conditions such as diabetes, cancers, schizophrenia or heart disease. Assessing the risks of these conditions allows you and your patients to compare overall disease risks among embryos and make decisions about which embryo(s) to prioritize for transfer.

SHARE

We have created an **educational video** for providers to share with patients, to help inform and Identify patients who may be Interested In this testing. It may be viewed at any time, on **www.embryohealthscore.com**.

1

ORDER

If the patient decides that that they would like to learn more and speak to us, order testing by submitting a completed **Test Requisition Form** and selecting PGT-P from the test menu. The patient will decide during this counseling whether they want the Embryo Health Scores or not.

2

COUNSEL

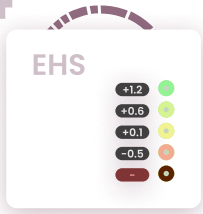
Genomic Prediction Clinical Laboratory will contact your patient to schedule a virtual pre-test genetic counseling session, to review the benefits, limitations and costs of PGT-P. Disease panels are based on self-reported ancestry, and will be reviewed during this session.

3

REPORT

Patients electing to receive Embryo Health Scores will be sent a saliva collection kit. PGT-P is reported using the same embryo biopsy used for PGT-A. No difference in procedure Is required. Reports are sent directly to the IVF provider. Patients are encouraged to contact our counsel with any questions following testing.

4



REFERENCES

To learn more about EHS and our publications, please visit www.lifeview.com.

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PGT-P

Genomic Prediction
Clinical Laboratory

Testing for polygenic disorders (PGT-P)

Polygenic disorders are diseases caused by genetic changes in more than one gene. Preimplantation genetic testing for polygenic disorders (PGT-P) is a genetic test performed on embryo biopsies, specifically designed to identify the risk of certain polygenic disorders in embryos. Examples of polygenic disorders available for screening include:

- **Schizophrenia**
- **Type 1 diabetes**
- **Type 2 diabetes**
- **Prostate cancer**
- **Heart attack**
- **Hypertension**
- **Breast cancer**
- **Basal cell carcinoma**
- **Malignant melanoma**
- **Testicular cancer**
- **Hypercholesterolemia**
- **Coronary artery disease**

What is the benefit of PGT-P?

LifeView™ PGT-P is used to identify embryo's lifetime risk of developing certain conditions. This testing is designed to screen several conditions at the same time. All of the designed to screen several conditions at the same time. All of the disease risks for each embryo are merged into a single number: the **Embryo Health Score** (EHS). The EHS score may be used to compare overall disease risk among embryos, to help you and your physician decide which embryo(s) to select for thansfer, and in which order. Embryos with higher embryo health scores are estimated to have lower over all disease risks.

All couples may benefit from LifeView™ PGT-P. Anyone may consider using LifeView™ PGT-P, especially if you already plan to unclude aneuploidy screening (PGT-A) in your IVF plan, or if you have a personal or a family history of a polygenic disorder such as type 1 diabetes, breast cancer or schizophrenia.

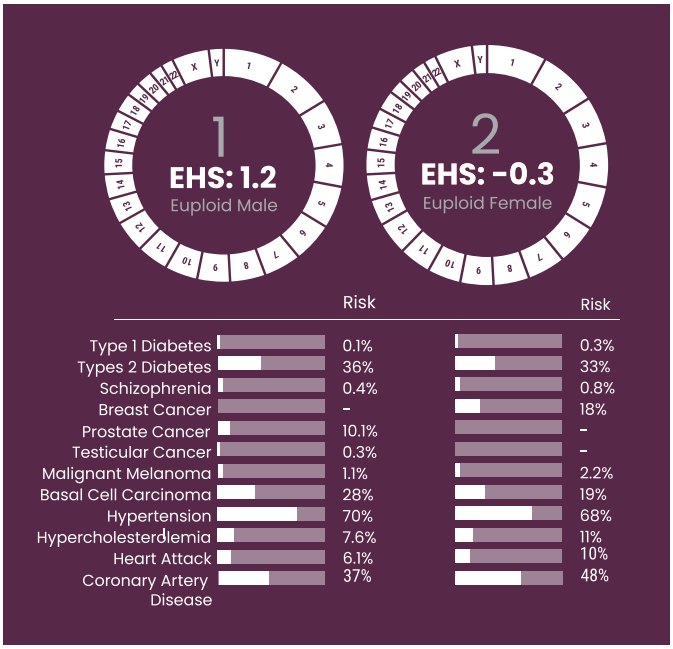
How accurate is LifeView™ PGT-P?

LifeView™ PGT-P is a screening tool used to help select wich embryo(s) to transfer, in which order. Selection among siblings using EHS ranking has been validated to reduce disease incidence by up to 72% for some conditions.

For detailed results specific to each condition and family history, visit: **www.lifeview.net**

What is the process of LifeView™ PGT-P?

Genetic counseling is included at no extra charge to review the process and detail benefits and limitations of LifeView™ PGT-P. Maternal and paternal saliva from each embryo. A report is sent to your physician within 14 days of receiving samples.



Is PGT-A included in LifeView™ PGT-P?

Yes. LifeView™ PGT-P includes aneuploidy screening, known as PGT-A, at no additional cost. PGT-A can be included without the need for any additional biopsies, samples or procedures.

Can LifeView™ PGT-P be expanded to include other types of testing?

LifeView™ PGT-P may also be expanded to include PGT-M (testing for structural rearrangements), or M2 (genetic miscarriage risk assessment of the embryo). Additional embryo). Additional embryo biopsies are not required.

Who can I ask questions about LifeView™?

Genetic counceling is included with LifeView™ at no extra charge. Ask your IVF team to refer you for a vitual genetic counseling session.

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Miscarriage Risk Assessment

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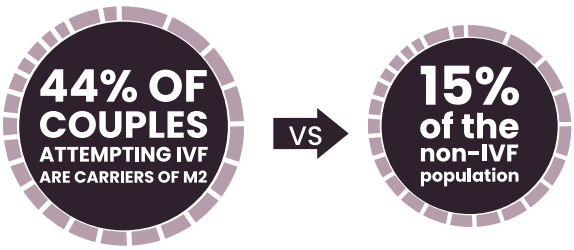
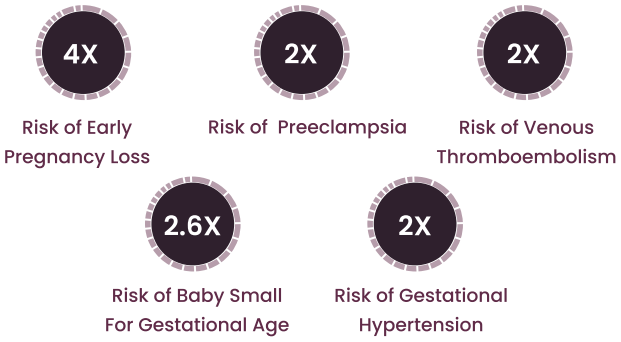
Clinical Laboratory

Genomic Prediction is conducting an observational study to determine how patients'M2 carrier status is associated with history of pregnancy complications, and the utilization and outcomes of interventions available to carrier couples. M2 is a genetic variant that increases the risk of placental mediated pregnancy loss and complications such as pre-eclampsia and miscarriage during pregnancy. Treatment may include the use of low molecular weight heparin (LMWH) injections during pregnancy or the use of a newly developed test to detect the M2 variant in embryos and select non-carrier embryos for transfer.



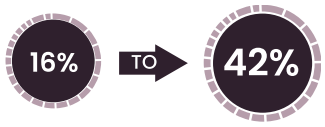
M2 CARRIERS HAVE



Participation in the study is fast and easy. Patients who are over the age of 18 and can provide a saliva sample from both the biological parents of the embryos are eligible to participate. Patients who elect to participate will have parental M2 carriertesting performed free of charge. A report will be issued to you with your results. As part of your study participation your medical history will be collected. For carrier couples, your decisions and outcomes of electing for LMWH treatment, use of the newly developed embryo testing, or no intervention will also be monitored and recorded. Patients who elect to use Preimplantation Genetic Testing (PGT) can obtain PGT for embryonic M2 carrier status without paying fees above traditional PGT-A (aneuploidy) costs.

Effective Interventions:

Couples identified with the M2 variant have a higher risk of miscarriage. Intervention has the potential to increase your chance of carrying to term from:



Intervention 1

Low molecular weight heparin* (daily injections)

* Fishel et al. EBioMedicine. 2016

LifeView™ M2

Intervention 2

M2 carriers can screen embryos for M2 status, as an alternative to drug treatment
Miscarriage risk from M2 comes equally from the mother and father.
M2 is expressed in the genome of the embryo. It is possible to choose an embryo with no M2, using LifeView preimplantation genetic testing (PGT).

LifeView™ PGT

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PGT-SR
For Structural Rearrangements

Genomic Prediction
Clinical Laboratory

Testing for structural rearrangements (PGT-SR)

Preimplantation genetic testing for structural rearrangements (PGT-SR) is a genetic test performed on embryo biopsies, specifically designed to screen embryos for extra or missing chromosome material associated with a parental structural rearrangement.

Who can benefit from PGT-SR?

PGT-SR is appropriate for individuals identified as having a balanced chromosome rearrangement. "Structural" or "chromosomal" rearrangements refer to chromosomal material that is ordered differently than usual. Individuals who are carriers of a balanced chromosome rearrangement have a higher chance of creating embryos with extra or missing genetic material; leading to fertility problems, pregnancy loss and health problems in an ongoing pregnancy.

How is this information helpful?

Screening embryos for extra or missing genetic material may help you and your doctor decide which embryos to select transfer, to increase the chance of a healthy pregnancy outcome.

Can LifeView™ PGT-SR be used for different kinds of structural rearrangements?

Yes. Genomic Prediction Clinical Laboratory has experience testing for different types of chromosome rearrangements, including:
- **Reciprocal translocations:** Chomosome material from two different chromosomes have swapped places.
- **Inversions:** Chromosome material is “flipped over” within a chromosome.
- **Robertsonian translocations:** Two different chromosomes are attached together.

Can LifeView™ distinguish between embryos with a normal karyotype and embryos that are positive for a balanced rearrangement

Yes. As long as we have data for comparison, it is possible to distinguish between embryos that inherited the balanced chromosome rearrangement identified in the parent and embryos that are negative for the rearrarangement. This is one of the ways that LifeView™ PGT-SR is superior to other PGT-SR options.

What is the process?

Prior to testing, we review your genetic reports (karyotypes) in order to confirm the testing plan. Maternal and paternal saliva samples are collected along with biopsy samples from each embryo. In some cases, saliva or other DNA samples from additional family members may be needed. Once all of the samples are recieved, a genetic report is generated and sent to your physician within 14 days.

How accurate is LifeView™ PGT-SR?

Diagnostic accuracy is greater than 99%.

Is aneuploidy (PGT-A) included in LifeView™?

Yes. All LifeView™ PGT-SR includes aneuploidy screening, or PGT-A, to screen for other forms of aneuploidy unrelated to the parental chromosome rearrangement. This screening is performed at no extra charge and without the need for additional embryo biopsy samples.

Can LifeView™ PGT-SR be expanded to include other types of testing?

Using the same sample, LiveView™ PGT-SR may also be expanded to include:
PGT-P: LifeView™ is the only platform validated to accurately predict each embryos lifetime risk for polygenic disorders; diseases influenced by variants in many genes, such as diabetes and certain cancers. Deciding which embryos to prioritize for transfer using LifeView™ PGT-P has been validated to reduce incidence of these disorders later in life.
PGT-M: Preimplantation genetic testing for monogenic disorders is uniquely designed for individuals or couples at risk of passing on a monogenic, or single gene, disorder.
M2: M2 is a genetic variant associated with increased risk of pregnancy complications and miscarriage. For couples with M2, LifeView™ can can be expanded to include M2 haplotype testing, to help you and your doctor make informed interventions to reduce the risk of miscarriage.

Who can I ask questions about LifeView™?

Genetic counseling is included with LifeView™ at no extra charge. Ask your IVF team to refer you for a virtual genetic counseling session.

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PGT-M
For Monogenic Disorders

Genomic Prediction
Clinical Laboratory

Testing for monogenic disorders (PGT-M)

Preimplantation genetic testing for monogenic disorders (PGT-M) is a genetic test performed on embryo biopsies, specifically designed to screen monogenic, or "single gene" disorders.
Tay-Sachs disease, sickle cell disease, cystic fibrosis, Duchenne muscular dystrophy and fragile-X syndrome are all examples of monogenic disorders.

Who can benefit from PGT-M?

Couples at risk of passing down a monogenic disorder may benefit from PGT-M.

- Couples with a child, or a personal or family history of a monogenic disorder.
- Couples identified as at-risk, through routine carrier screening.
- Couples interested in HLA matching.

How does the result help?

Seeing which embryos test positive for a disorder helps you and your doctor decide which embryos to transfer, and in which order.

How does LifeViewTM PGT-M work, step by step?

1. Prior to testing, we review your genetic reports, along with your medical and family history, in order to create a plan to detect the condition(s) requested for testing. No two tests are exactly alike.
2. Saliva samples are collected from each member of the couple. In some cases, saliva or other DNA samples from additional family members may be needed.
3. Once all of the needed samples are received, the testing plan is confirmed, and your IVF center is notified that we are able to proceed with receiving biopsies for testing.
4. After embryo biopsy samples are sent to our laboratory, it takes 14 days to complete the analysis.
5. A PGT-M genetic report is generated by our laboratory, and sent to your preferred clinician.

How accurate is LifeViewTM PGT-M?

LifeViewTM is among the most accurate PGT-M available. Diagnostic accuracy ranges exceed 97-99%.

There is more than one monogenic disorder in my family. Can you test for multiple conditions

Yes. As long as we can design a testing plan for each individual monogenic disorder that you wish to be tested, there is no limit to the number of conditions evaluated on a single sample.

Is aneuploidy (PGT-A) included in LifeViewTM?

Yes. All LifeViewTM PGT-M includes aneuploidy screening, or PGT-A, at no extra charge. This screening is added without the need for additional embryo biopsy samples.

Can LifeViewTM PGT-M be expanded to include other types of testing?

Using the same samples, with no additional embryo biopsies, LifeViewTM PGT-M may also be expanded to include:
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PGT-SR: LifeViewTM for structural rearrangements identifies extra or missing chromosomal material related to a parental chromosome rearrangement.
M2: M2 is a genetic variant associated with increased risk of pregnancy complications and miscarriage. For couples with M2, LifeViewTM can can be expanded to include M2 haplotype testing, to help you and your doctor make informed interventions to reduce the risk of miscarriage.

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Scientific Validations

Genomic Prediction
Clinical Laboratory

PREIMPLANTATION GENETIC TESTING FOR POLYGENIC DISEASE RISK

REPRODUCTION
ANNIVERSARY REVIEW

Nathan Treff, Diego Marin, Louis Lello, Stephen Hsu, Laurent CAM Tellier

Since its introduction to clinical practice, preimplantation genetic testing (PGT) has become a standard of care for couples at risk of having children with monogenic disease, and for chromosomal aneuploidy to improve outcomes for patients with infertility. The primary objective of PGT is to reduce the risk of miscarriage and genetic disease and to improve the success of infertility treatment with the delivery of a healthy child. Until recently, the application of PGT to more common but complex polygenic disease was not possible, as the genetic contribution to polygenic disease has been difficult to determine, and the concept of embryo selection across multiple genetic loci has been difficult to comprehend. Several achievements, including the ability to obtain accurate, genome-wide genotypes of the human embryo, and the development of population level biobanks have now made PGT for polygenic disease risk applicable in clinical practice. With the rapid advances in embryonic polygenic risk scoring, diverse considerations beyond technical capability have been introduced.

SIBLING VALIDATION OF POLYGENIC RISK SCORES AND COMPLEX TRAIT PREDICTION

Trends in Genetics

Louis Lello, Timothy G. Raben & Stephen D. H. Hsu

We test 26 polygenic predictors using tens of thousands of genetic siblings from the UKBiobank (UKB), for whom we have SNP genotypes, health status, and phenotype information in late adulthood. Siblings have typically experienced similar environments during childhood, and exhibit negligible population stratification relative to each other. Therefore, the ability to predict differences in disease risk or complex trait values between siblings is a strong test of genomic prediction in humans. We compare validation results obtained using non-sibling subjects to those obtained among siblings and find that typically most of the predictive power persists in between-sibling designs. In the case of disease risk we test the extent to which higher polygenic risk score (PRS) identifies the affected sibling, and also compute Relative Risk Reduction as a function of risk score threshold. For quantitative traits we examine between-sibling differences in trait values as a function of predicted differences, and compare to performance in non-sibling pairs. Example results: Given 1 sibling with normal-range PRS score (<84 percentile, < + 1 SD) and 1 sibling with high PRS score (top few percentiles, i.e. > + 2 SD), the predictors identify the affected sibling about 70–90% of the time across a variety of disease conditions, including Breast Cancer, Heart Attack, Diabetes, etc. 55–65% of the time the higher PRS sibling is the case. For quantitative traits such as height, the predictor correctly identifies the taller sibling roughly 80 percent of the time when the (male) height difference is 2 inches or more.

GENETIC ARCHITECTURE OF COMPLEX TRAITS ANDDISEASE RISK PREDICTORS

SCIENTIFIC REPORTS
nature research

Soke Yuen Yong, TimothyG.Raben Louis Lello & Stephen D. H. Hsu

Genomic prediction of complex human traits (e.g., height, cognitive ability, bone density) and disease risks (e.g., breast cancer, diabetes, heart disease, atrial fibrillation) has advanced considerably in recent years. Using data from the UKBiobank, predictors have been constructed using penalized algorithms that favor sparsity: i.e., which use as few genetic variants as possible. We analyze the specific genetic variants (SNPs) utilized in these predictors, which can vary from dozens to as many as thirty thousand. We find that the fraction of SNPs in or near genic regions varies widely by phenotype. for the majority of disease conditions studied, a large amount of the variance is accounted for by Snps outside of coding regions. the state of these Snps cannot be determined from exome- sequencing data. this suggests that exome data alone will miss much of the heritability for these traits—i.e., existing pRS cannot be computed from exome data alone. We also study the fraction of Snps and of variance that is in common between pairs of predictors. the DnA regions used in disease risk predictors so far constructed seem to be largely disjoint (with a few interesting exceptions), suggesting that individual genetic disease risks are largely uncorrelated. it seems possible in theory for an individual to be a low-risk outlier in all conditions simultaneously.

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Scientific Validations

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PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY: A REVIEW OF PUBLISHED BLASTOCYST REANALYSIS CONCORDANCE DATA

PRENATAL DIAGNOSIS
WILEY

Diego Marin, Jia Xu, Nathan R. Treff

Preimplantation genetic testing for aneuploidy (PGT-A) reduces miscarriage risk, increases the success of IVF, shortens time to pregnancy, and reduces multiple gesta- tion rates without compromising outcomes. The progression of PGT-A has included common application of next-generation sequencing (NGS) from single nucleotide polymorphism microarray, quantitative real-time PCR, and array comparative hybridi- zation platforms of analysis. Additional putative advances in PGT-A capability include classifying embryos as mosaic and predicting the presence of segmental imbalance. A critical component in the process of technical validation of these advancements involves evaluation of concordance between reanalysis results and initial testing results. While many independent studies have investigated the concordance of results obtained from the remaining embryo with the original PGT-A diagnosis, com- pilation and systematic analysis of published data has not been performed. Here, we review results from 26 primary research articles describing concordance in 1271 human blastocysts from 2260 pairwise comparisons. Results illustrate significantly higher discordance from PGT-A methods which utilize NGS and include prediction of mosaicism or segmental imbalance. These results suggest caution when considering new iterations PGT-A.

THE ''MOSAIC'' EMBRYO: MISCONCEPTIONS AND MISINTERPRETATIONS IN PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY

Fertility and Sterility. asrm

Nathan R. Treff, Ph.D. and Diego Marin, Ph.D.

Preimplantation genetic testing for aneuploidy (PGT-A) remains one of the most controversial topics in reproductive medicine. With more than 40% of in vitro fertilization cycles in the United States reportedly involving PGT, both those in favor of and those opposed to PGT-A have significant interest in the efficacy of PGT-A. Ongoing issues include what patient population, if any, benefits from PGT-A, the true frequency of chromosomal mosaicism, whether embryonic aneuploidies self-correct, and how practitioners manage embryos designated as "mosaic." This review addresses several misconceptions and misinterpretations of data surrounding the genetic analysis and prediction of mosaicism in the preimplantation embryo.

PREIMPLANTATION GENETIC TESTING FOR POLYGENIC DISEASE RELATIVE RISK REDUCTION: EVALUATION OF GENOMIC INDEX PERFORMANCE IN 11, 883 ADULT SIBLING PAIRS

GCAT TACG GCAT genes

Nathan R. Treff, Jennifer Eccles, Diego Marin, Edward Messick, Louis Lello, Jessalyn Gerber, Jia Xu and Laurent C. A. M. Tellier

Preimplantation genetic testing for polygenic disease risk (PGT-P) represents a new tool to aid in embryo selection. Previous studies demonstrated the ability to obtain necessary genotypes in the embryo with accuracy equivalent to in adults. When applied to select adult siblings with known type I diabetes status, a reduction in disease incidence of 45–72% compared to random selection was achieved. This study extends analysis to 11,883 sibling pairs to evaluate clinical utility of embryo selection with PGT-P. Results demonstrate simultaneous relative risk reduction of all diseases tested in parallel, which included diabetes, cancer, and heart disease, and indicate applicability beyond patients with a known family history of disease.

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A NOVEL TEST FOR ANNEXIN A5 M2 HAPLOTYPING IN VITRO FERTILIZATION PATIENTS AND PREIMPLANTATION EMBRYOS

Fertility and Sterility. asrm

Bhavini Rana, B.A., Raymond Zimmerman, M.S., Diego Marin, Ph.D., Jia Xu, Ph.D., Edward Messick, M.S., Simon Fishel, Ph.D., and Nathan Treff, Ph.D

Objective: *To develop a test for evaluating the annexin A5 M2 haplotypein in vitrofertilization patients and preimplantation embryos.*
Design: *Test performance was measured by comparing Sanger sequencing of parental blood DNA and quantitative real-time polymerase chain reaction (qPCR) of saliva DNA, 3 fibroblast cell line 7-cell aliquots and their corresponding purified DNA, 123 trophectoderm biopsy samples, and DNA isolated from 1 embryonic stem cell line along with the Mendelian inheritance expectations, embryo Sanger sequencing, and single-nucleotide polymorphism (SNP) microarray-based linkage analysis.*
Setting: Preimplantation genetic testing laboratory research on IVF patient and embryo DNA.
Patient(s): An assay was developed for the detection of the M2 haplotype on saliva samples of 6 in vitro fertilization patients. In addi- tion, 13 patients who underwent preimplantation genetic testing with data on parental and embryo biopsy DNA available for research use were evaluated.

ALLELE-SPECIFIC CHROMOSOME REMOVAL AFTER CAS9 CLEAVAGE IN HUMAN EMBRYOS

Cell

Michael V Zuccaro, Jia Xu, Carl Mitchell, Diego Marin, Raymond Zimmerman, Bhavini Rana, Everett Weinstein, Rebeca T King, Katherine L Palmerola, Morgan E Smith, Stephen H Tsang, Robin Goland, Maria Jasin, Rogerio Lobo, Nathan Treff, Dieter Egli

Correction of disease-causing mutations in human embryos holds the potential to reduce the burden of inherited genetic disorders and improve fertility treatments for couples with disease-causing mutations in lieu of embryo selection. Here, we evaluate repair outcomes of a Cas9-induced double-strand break (DSB) introduced on the paternal chromosome at the EYS locus, which carries a frameshift mutation causing blindness. We show that the most common repair outcome is microhomology-mediated end joining, which occurs during the first cell cycle in the zygote, leading to embryos with non-mosaic restoration of the reading frame. Notably, about half of the breaks remain unrepaired, resulting in an undetectable paternal allele and, after mitosis, loss of one or both chromosomal arms. Correspondingly, Cas9 off-target cleavage results in chromosomal losses and hemizygous indels because of cleavage of both alleles. These results demonstrate the ability to manipulate chromosome content and reveal significant challenges for mutation correction in human embryos.

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EDITORIAL: CAUSES OF OOCYTE ANEUPLOIDY AND INFERTILITY IN ADVANCED MATERNAL AGE AND EMERGING THERAPEUTIC APPROACHES

frontiers

Lori R. Bernstein and Nathan R. Treff

Women of advanced maternal age (AMA, age ≥ 35) experience elevated rates of infertility, miscarriages and trisomic pregnancies (Ubaldi et al.). They also exhibit diminished ovarian reserve (DOR), as well as poor ovarian responses (POR) to gonadotropin stimulation in assisted reproduction (Fuentes et al.). Oocyte and embryo aneuploidy are primary causes of declining oocyte and embryo quality and diminished rates of pregnancy and livebirth in AMA women. Mechanisms that underlie these processes are thought to include cohesin dysfunction, telomere shortening, spindle instability, aberrant checkpoint control, reductions in rates of preimplantation embryo development to the blastocyst stage, age-related hormonal aberrations, and mitochondrial dysfunction (Ubaldi et al.; Fuentes et al.) (1). Some factors that reduce oocyte quality and embryo quality in AMA women may also be at play in the pathogenesis of endometriosis (Máté et al.). Current and emerging technological advances continue to be developed to treat and even prevent oocyte aneuploidy in patients with diminished oocyte and embryo quality. Here present an editorial summary of these themes in this article collection, along with some recent data drawn from additional relevant papers.

ISN'T IT TIME TO STOP CALLING PREIMPLANTATION EMBRYOS ''MOSAIC''?

Fertility and Sterility asrm

Richard J. Paulson, M.D., M.S, Nathan R. Treff, Ph.D., H.C.L.D.

This August, the long-awaited Practice Committee opinion on the management of mosaic results from preimplantation genetic testing for aneuploidies (PGT-A) was published (1). It is an impressive body of work that will be helpful to clini- cians and patients who are dealing with the difficult decision of which embryos to transfer. The opinion is overall very well balanced and carefully written. However, the authors stopped short of calling for the abandonment of the term, ''mosaic'' when referring to ''intermediate copy number'' of individual chromosomes. The latter term is more accurate, and we pro- pose that it should be used in place of the inaccurate and, arguably, misleading term ''mosaic.''

EMBRYO SCREENING FOR POLYGENIC DISEASE RISK: RECENT ADVANCES AND ETHICAL CONSIDERATIONS

GCAT TACG GCAT genes

by Laurent C. A. M. Tellier, Jennifer Eccles, Nathan R. Treff, Louis Lello, Simon Fishel and Stephen Hsu

Machine learning methods applied to large genomic datasets (such as those used in GWAS) have led to the creation of polygenic risk scores (PRSs) that can be used identify individuals who are at highly elevated risk for important disease conditions, such as coronary artery disease (CAD), diabetes, hypertension, breast cancer, and many more. PRSs have been validated in large population groups across multiple continents and are under evaluation for widespread clinical use in adult health. It has been shown that PRSs can be used to identify which of two individuals is at a lower disease risk, even when these two individuals are siblings from a shared family environment. The relative risk reduction (RRR) from choosing an embryo with a lower PRS (with respect to one chosen at random) can be quantified by using these sibling results. New technology for precise embryo genotyping allows more sophisticated preimplantation ranking with better results than the current method of selection that is based on morphology. We review the advances described above and discuss related ethical considerations.

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GENOMIC PREDICTION OF 16 COMPLEX DISEASE RISKS INCLUDING HEART ATTACK, DIABETES, BREAST AND PROSTATE CANCER

SCIENTIFIC REPORTS
nature research

Louis Lello, Timothy G. Raben, Soke Yuen Yong, Laurent C. A. M. Tellier & Stephen D. H. Hsu

We construct risk predictors using polygenic scores (PGS) computed from common Single Nucleotide Polymorphisms (SNPs) for a number of complex disease conditions, using L1-penalized regression (also known as LASSO) on case-control data from UK Biobank. Among the disease conditions studied are Hypothyroidism, (Resistant) Hypertension, Type 1 and 2 Diabetes, Breast Cancer, Prostate Cancer, Testicular Cancer, Gallstones, Glaucoma, Gout, Atrial Fibrillation, High Cholesterol, Asthma, Basal Cell Carcinoma, Malignant Melanoma, and Heart Attack. We obtain values for the area under the receiver operating characteristic curves (AUC) in the range ~0.58–0.71 using SNP data alone. Substantially higher predictor AUCs are obtained when incorporating additional variables such as age and sex. Some SNP predictors alone are sufficient to identify outliers (e.g., in the 99th percentile of polygenic score, or PGS) with 3–8 times higher risk than typical individuals. We validate predictors out-of-sample using the eMERGE dataset, and also with different ancestry subgroups within the UK Biobank population. Our results indicate that substantial improvements in predictive power are attainable using training sets with larger case populations. We anticipate rapid improvement in genomic prediction as more case-control data become available for analysis.

VALIDATION OF CONCURRENT PREIMPLANTATION GENETIC TESTING FOR POLYGENIC AND MONOGENIC DISORDERS, STRUCTURAL REARRANGEMENTS, AND WHOLE AND SEGMENTAL CHROMOSOME ANEUPLOIDY WITH A SINGLE UNIVERSAL PLATFORM

EUROPEAN JOURNAL OF MEDICAL GENETICS

Nathan R. Treff, Raymond Zimmerman , Elan Bechor, Jeff Hsu , Bhavini Rana, Jens Jensen, Jeremy Lia, Artem Samoilenko, William Mowrey, James Van Alstine, Mark Leondires, Kathy Miller, Erica Paganetti, Louis Lello, Steven Avery, Stephen Hsu, Laurent C. A. Melchior Tellier

Preimplantation genetic testing (PGT) has been successfully applied to reduce the risk of miscarriage, improve IVF success rates, and prevent inheritance of monogenic disease and unbalanced translocations. The present study provides the first method capable of simultaneous testing of aneuploidy (PGT-A), structural rearrangements (PGT-SR), and monogenic (PGT-M) disorders using a single platform. Using positive controls to establish performance characteristics, accuracies of 97 to >99% for each type of testing were observed. In addition, this study expands PGT to include predicting the risk of polygenic disorders (PGT-P) for the first time. Performance was established for two common diseases, hypothyroidism and type 1 diabetes, based upon availability of positive control samples from commercially available repositories. Data from the UK Biobank, eMERGE, and T1DBASE were used to establish and validate SNP-based predictors of each disease (7,311 SNPs for hypothyroidism and 82 for type 1 diabetes). Area under the curve of disease status prediction from genotypes alone were 0.71 for hypothyroidism and 0.68 for type 1 diabetes. The availability of expanded PGT to evaluate the risk of polygenic disorders in the preimplantation embryo has the potential to lower the prevalence of common genetic disease in humans.

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UTILITY AND FIRST CLINICAL APPLICATION OF SCREENING EMBRYOS FOR POLYGENIC DISEASE RISK REDUCTION

frontiers

Nathan R. Treff, Jennifer Eccles, Lou Lello, Elan Bechor, Jeffrey Hsu, Kathryn Plunkett, Raymond Zimmerman, Bhavini Rana, Artem Samoilenko, Steven Hsu and Laurent C. A. M. Tellier

For over 2 decades preimplantation genetic testing (PGT) has been in clinical use to reduce the risk of miscarriage and genetic disease in patients with advanced maternal age and risk of transmitting disease. Recently developed methods of genome-wide genotyping and machine learning algorithms now offer the ability to genotype embryos for polygenic disease risk with accuracy equivalent to adults. In addition, contemporary studies on adults indicate the ability to predict polygenic disorders with risk equivalent to monogenic disorders. Existing biobanks provide opportunities to model the clinical utility of polygenic disease risk reduction among sibling adults. Here, we provide a mathematical model for the use of embryo screening to reduce the risk of type 1 diabetes. Results indicate a 45–72% reduced risk with blinded genetic selection of one sibling. The first clinical case of polygenic risk scoring in human preimplantation embryos from patients with a family history of complex disease is reported. In addition to these data, several common and accepted practices place PGT for polygenic disease risk in the applicable context of contemporary reproductive medicine. In addition, prediction of risk for PCOS, endometriosis, and aneuploidy are of particular interest and relevance to patients with infertility and represent an important focus of future research on polygenic risk scoring in embryos.

EDITORIAL: CAUSES OF OOCYTE ANEUPLOIDY AND INFERTILITY IN ADVANCED MATERNAL AGE AND EMERGING THERAPEUTIC APPROACHES

Fertility and Sterility asrm

Lori R. Bernstein and Nathan R. Treff

Women of advanced maternal age (AMA, age ≥ 35) experience elevated rates of infertility, miscarriages and trisomic pregnancies (Ubaldi et al.). They also exhibit diminished ovarian reserve (DOR), as well as poor ovarian responses (POR) to gonadotropin stimulation in assisted reproduction (Fuentes et al.). Oocyte and embryo aneuploidy are primary causes of declining oocyte and embryo quality and diminished rates of pregnancy and livebirth in AMA women. Mechanisms that underlie these processes are thought to include cohesin dysfunction, telomere shortening, spindle instability, aberrant checkpoint control, reductions in rates of preimplantation embryo development to the blastocyst stage, age-related hormonal aberrations, and mitochondrial dysfunction (Ubaldi et al.; Fuentes et al.) (1). Some factors that reduce oocyte quality and embryo quality in AMA women may also be at play in the pathogenesis of endometriosis (Máté et al.). Current and emerging technological advances continue to be developed to treat and even prevent oocyte aneuploidy in patients with diminished oocyte and embryo quality. Here present an editorial summary of these themes in this article collection, along with some recent data drawn from additional relevant papers.

POLYGENIC RISK SCORES, PGT-P, AND IVF: SIBLING AND NON-EUROPEAN ANCESTRY VALIDATIONS.

Fertility and Sterility asrm

Louis Lello, PhD; Laurent C. A. M. Tellier; BS2 Michigan State University, East Lansing, MI; Genomic Prediction, North Brunswick, NJ.

Polygenic Risk Scores (PRS) are under intense investiga- tion by research groups all around the world. Large genomic datasets and ma- chine learning methods have produced predictors for common disease conditions such as coronary artery disease, diabetes, schizophrenia, and breast cancer. The objective of this study is to further validate PRS and related health and longevity indices, using sibling (family) cohorts and non-European (African, South Asian, and East Asian) ancestry groups.

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ACCURATE GENOMIC PREDICTION OF MOSAICISM THROUGH CELL DIVISION ORIGIN OF ANEUPLOIDY ANALYSIS IN THE PREIMPLANTATION EMBRYO

Fertility and Sterility. asrm

Diego Marin, PhD,1 Bhavini Rana, BA, Heather Garnsey, MS, Adrienne Faunce, BS, John Garrisi, PhD, Robert J. Mendola, MSc, TS (ABB), Jia Xu, PhD, Nathan R. Treff, PhD, HCLD Genomic Prediction, North Brunswick, NJ; Institute for Reproductive Medicine and Science, Livingston, NJ.

Simple intermediate copy number thresholds have proven to provide poor predictive value for diagnosing preimplantation mosaicism (both analytically and clinically). This study aims to validate a method to determine the cell division origin of aneuploidy from a single trophectoderm biopsy, and its ability to accurately predict mosaicism in the remaining em- bryo.

DNA DOUBLE-STRAND BREAKS FREQUENTLY CAUSE CHROMOSOME LOSS IN HUMAN EMBRYOS

Fertility and Sterility. asrm

Jenna M. Turocy, M.D.; Michael V. Zuccaro, PhD; Diego Marin, PhD; Jia Xu, PhD; Nathan R. Treff, PhD, HCLD; Dieter Egli, PhD; Columbia University Fertility Center, New York, NY; Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, New York, NY; Genomic Prediction; Genomic Prediction, Inc., North Brunswick, NJ; Genomic Prediction, North Brunswick, NJ; 61150 St. Nicholas Avenue, New York, NY.

Genome editing by DNA double-strand breaks (DSB) is currently being investigated as a tool to treat or even prevent heritable dis- eases. However, DNA repair mechanisms in the human embryo remain poorly understood and may result in small genetic variations such as indels, as well as large unwanted changes including loss of heterozygosity or the loss of a whole chromosome. Our study aims to determine outcomes of DNA repair after pericentromeric Cas9 cleavage in human preimplantation em- bryos.

GENETIC ANALYSIS OF THE ANNEXIN A5 M2 HAPLOTYPE AND ASSOCIATION WITH PREGNANCY COMPLICATIONS IN 500,000 INDIVIDUALS

Fertility and Sterility. asrm

Bhavini Rana, BA, Diego Marin, PhD, Jia Xu, PhD, Louis Lello, PhD, Simon Fishel, PhD, Nathan R. Treff, PhD, HCLD Genomic Prediction, North Brunswick, NJ; Michigan State University, East Lansing, MI; CARE Fertility Group, Nottingham, United Kingdom.

Meta-analysis has demonstrated a strong association be- tween the Annexin A5 M2 haplotype and pregnancy complications across multiple small cohorts. This study evaluated the validity of M2 imputa- tion from genome-wide DNA array data and investigates the association of M2 haplotypes with pregnancy complications reported in a large biobank.

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EXOME SEQUENCING LINKS CEP120 MUTATION TO MATERNALLY DERIVED ANEUPLOID CONCEPTION RISK

human reproduction

Katarzyna M. Tyc, Warif El Yakoubi, Aishee Bag, Jessica Landis, Yiping Zhan, Nathan R. Treff, Richard T. Scott, Xin Tao, Karen Schindler, Jinchuan Xing

Study question: What are the genetic factors that increase the risk of aneuploid egg production?
Summary answer: A non-synonymous variant rs2303720 within centrosomal protein 120 (CEP120) disrupts female meiosis in vitro in mouse.
What is known already: The production of aneuploid eggs, with an advanced maternal age as an established contributing factor, is the major cause of IVF failure, early miscarriage and developmental anomalies. The identity of maternal genetic variants contributing to egg aneuploidy irrespective of age is missing.
Study design, size, duration: Patients undergoing fertility treatment (n = 166) were deidentified and selected for whole-exome sequencing.
Participants/materials, setting, methods: Patients self-identified their ethnic groups and their ages ranged from 22 to 49 years old. The study was performed using genomes from White, non-Hispanic patients divided into controls (97) and cases (69) according to the number of aneuploid blastocysts derived during each IVF procedure. Following a gene prioritization strategy, a mouse oocyte system was used to validate the functional significance of the discovered associated genetic variants.
Main results and the role of chance: Patients producing a high proportion of aneuploid blastocysts (considered aneuploid if they missed any of the 40 chromatids or had extra copies) were found to carry a higher mutational burden in genes functioning in cytoskeleton and microtubule pathways. Validation of the functional significance of a non-synonymous variant rs2303720 within Cep120 on mouse oocyte meiotic maturation revealed that ectopic expression of CEP120:p.Arg947His caused decreased spindle microtubule nucleation efficiency and increased incidence of aneuploidy.
Limitations, reasons for caution: Functional validation was performed using the mouse oocyte system. Because spindle building pathways differ between mouse and human oocytes, the defects we observed upon ectopic expression of the Cep120 variant may alter mouse oocyte meiosis differently than human oocyte meiosis. Further studies using knock-in 'humanized' mouse models and in human oocytes will be needed to translate our findings to human system. Possible functional differences of the variant between ethnic groups also need to be investigated.
Wider implications of the findings: Variants in centrosomal genes appear to be important contributors to the risk of maternal aneuploidy. Functional validation of these variants will eventually allow prescreening to select patients that have better chances to benefit from preimplantation genetic testing.
Study funding/competing interest(s): This study was funded through R01-HD091331 to K.S. and J.X. and EMD Serono Grant for Fertility Innovation to N.R.T. N.R.T. is a shareholder and an employee of Genomic Prediction.

COMPLEX-TRAIT PREDICTION IN THE ERA OF BIG DATA

Cell

Gustavo de los Campos, Ana Ines Vazquez, Stephen Hsu and Louis Lello

Accurate prediction of complex traits requires using large number of DNA-variants. Advances in statistical and machine learning methodology enable the identification of complex patterns in high-dimensional settings. However, training these highly-parameterized methods requires very large data sets. Until recently, such data sets were not available. But the situation is changing rapidly as very large biomedical data sets comprising individual genotype-phenotype data for hundreds of thousands of individuals become available in public and private domains. We argue that the convergence of advances in methodology and the advent of Big Genomic Data will enable unprecedented improvements in complex trait prediction; we review theory and evidence supporting our claim and discuss challenges and opportunities that Big Data will bring to complex-trait prediction.

PGT-P Risk Reduction

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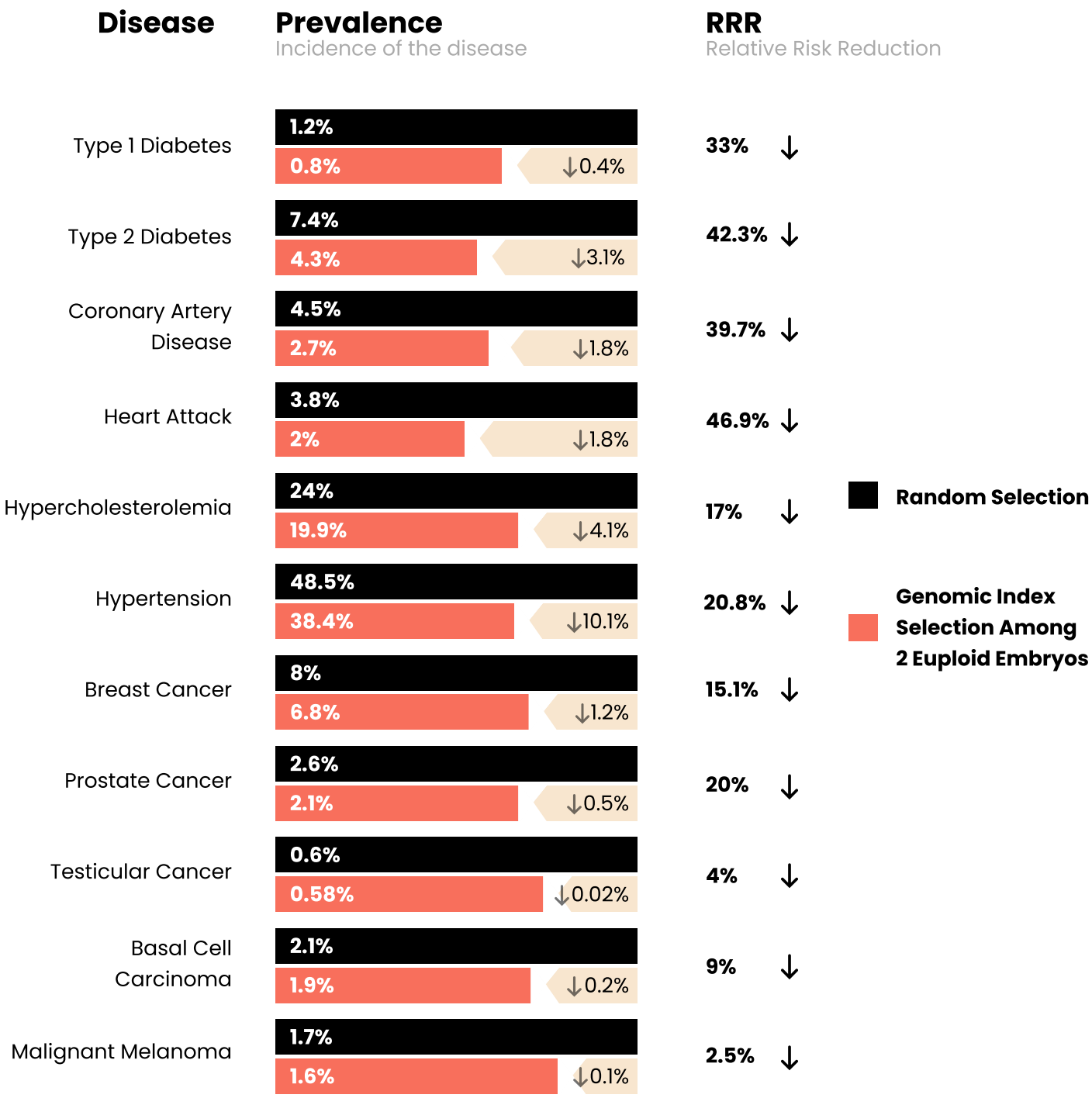

Treff et al, *Preimplantation Genetic Testing for Polygenic Disease Relative Risk Reduction: Evaluation of Genomic Index Performance in 11,883 Adult Sibling Pairs,* 2020, Genes

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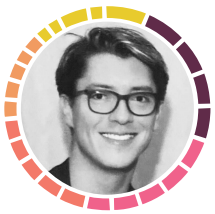

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