Pre-Implantation Genetic Screening (PGS) is a technique that counts the number of chromosomes present in embryos before they are transferred to the womb. The embryos are created during In Vitro Fertilisation (IVF) treatment. The aim of PGS is to increase the likelihood of having a baby by transferring embryos that have the correct number of chromosomes and are therefore deemed genetically balanced. By performing PGS on IVF embryos we aim to do one or more of the following:

- Lead to a higher number of babies born in the first or second cycle of IVF treatment.
- Reduce the risk of miscarriage
- Reduce the risk of having an abnormal pregnancy
- Reduce the risk of having a child with a chromosome abnormality, such as Downs Syndrome.
- Reduce the number of multiple pregnancies by transferring a SINGLE "genetically competent" embryo
- Provide useful diagnostic information regarding the likelihood of IVF success
- A quicker time to a successful outcome of IVF
- Reduced overall financial and emotional costs of achieving a viable pregnancy
- Reduce the number of stored embryos that are genetically imbalanced and therefore unlikely to result in viable pregnancies.

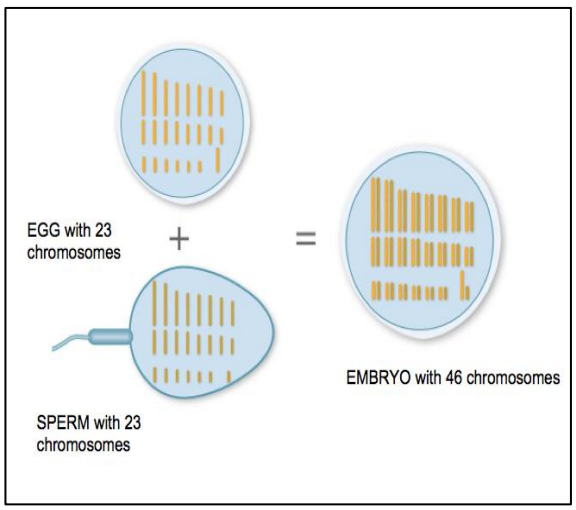
The following pages will provide information:

- How chromosome errors occur in embryos
- How PGS testing is performed at CARE
- The Benefits, Risks and Limitations of PGS
- Practical points relating to PGS

Introduction

Chromosome Abnormalities in Embryos

Chromosomes are tiny string-like structures that contain genes. Genes determine traits like eye and hair colour and direct growth and development of every part of the body. Typically, each person has 46 chromosomes in every cell arranged in 23 pairs. The diagram below shows the number of chromosomes in a mature egg and mature sperm combining to produce the first cell of an embryo.



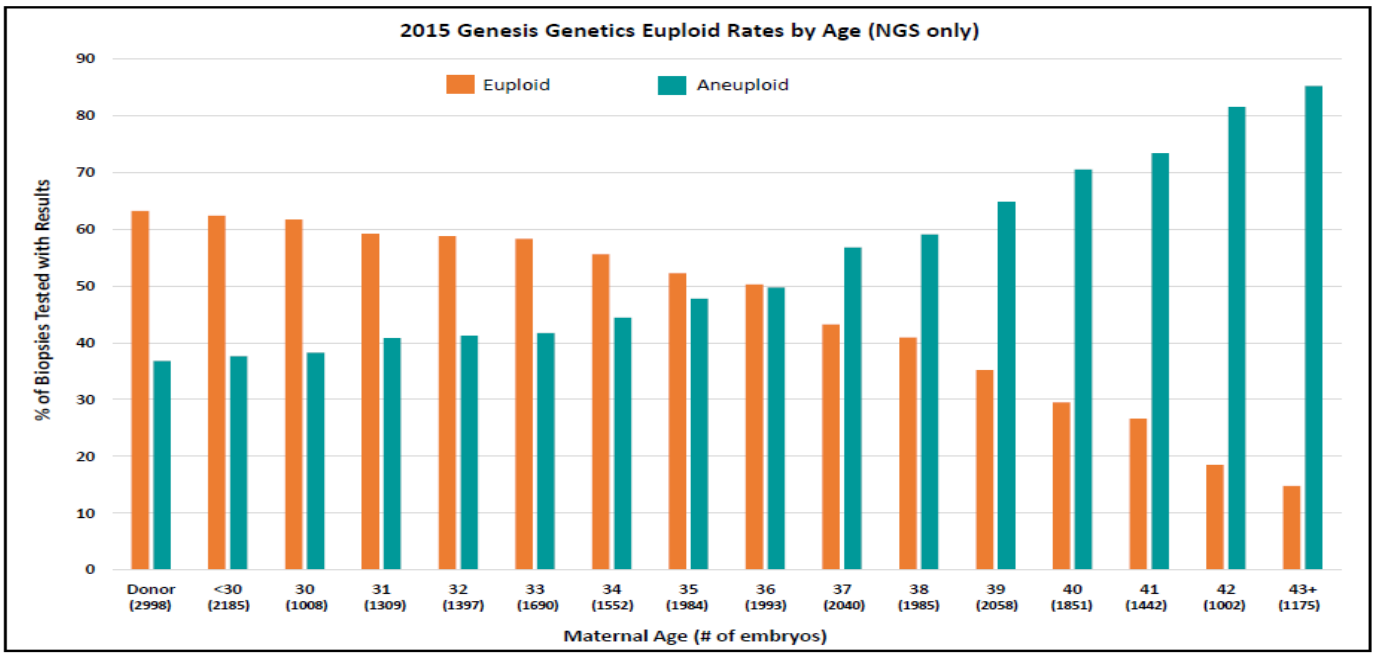
Embryos with the correct number of chromosomes (46) are genetically balanced and referred to as "euploid" or normal. If a mistake occurs when the egg or sperm cells mature resulting in missing or extra chromosomes, the embryo created from that egg or sperm would have an incorrect number of chromosomes and would be genetically unbalanced. This is a condition called aneuploidy (pronounced annu-ploy-dee). These embryos are referred to as aneuploid or abnormal.

Issues can occur in aneuploid embryos because there is either too much or too little genetic material present. These embryos will often not implant or may implant and then miscarry. There is a low chance that aneuploid embryos could also result in a pregnancy with abnormalities.

What causes aneuploidy?

Aneuploidy is natural process that happens mainly to egg cells as women age. Women are born with all their eggs; they do not make any more. As women age; their eggs age with them. From puberty, every month as an egg matures in the ovary, it undergoes a change to prepare for the reduction in the numbers of chromosomes from 46 to 23 during fertilisation. Sperm cells in the testes undergo a similar process to reduce their chromosome number from 46 to 23. A mistake in this division can produce eggs or sperm with too many or too few chromosomes.

It is well known there is an increased risk of miscarrying, an increased chance of Downs Syndrome and a decreasing likelihood of having a baby as women get older. With the latest PGS studies and our increasing understanding of the effect of age on fertility we now know that aneuploidy in embryos is much higher than originally thought. Approximately half (50%) of embryos from women aged 35-36 have chromosome aneuploidies, two thirds (66%) of embryos from women age 39 have chromosome aneuploidies and over three quarters (75%) of embryos from women aged 40-42 are aneuploid. The picture below shows embryo aneuploid and euploid rate by maternal age.



Graphic courtesy of Genesis Genetics International

There is growing evidence that PGS can significantly increase pregnancy and birth rates for women even below the age of 35 following single embryo transfer of a chromosomally normal embryo. Consequently, more patients are considering PGS as an add-on to routine IVF at an earlier age. A woman aged 30 has a good chance of pregnancy with an average of 62% of embryos being genetically balanced, however approximately 38% will be genetically unbalanced. This compares with a women aged 40 where the genetically balanced embryos are approximately 30% with 70% aneuploid.

Generally speaking, embryos with a missing chromosome (monosomy) or chromosomes (monosomies) will usually cease to grow before implanting into the womb. There is one exception, Turner Syndrome (monosomy X). Only a minority of embryos with an extra chromosome (trisomy) will implant and most will result in a miscarriage and few can potentially go on to full term resulting in eg Down syndrome (extra 21), Edward syndrome (extra 18), Patau Syndrome (extra 13) and Klinefelter Syndrome (extra X).

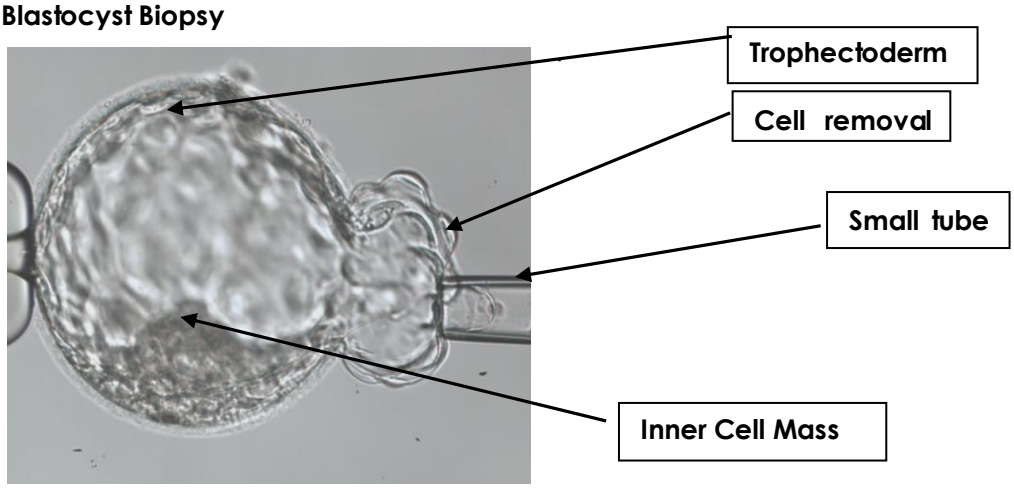
The incidence of aneuploidy does not affect men in the same way. This is mainly because sperm are continually made throughout a man's lifetime, on a 3 monthly replenishing cycle. Sperm are therefore relatively 'young' cells and the genetic integrity of the cell with respect to the number of chromosomes, is usually intact. Please note: if sperm parameters such as motility, count and shape are much lower than expected, this may have an effect on sperm aneuploidy. Your consultant will be able to advise you if further tests are required before treatment.

The aim of PGS, screening for aneuploidy, is to identify and select genetically balanced embryos for transfer to achieve viable pregnancies, reduce the number of pregnancy losses, and reduce the number of pregnancies or children affected by a chromosome abnormality.

HOW IS PGS PERFORMED AT CARE?

Following fertilisation the single embryo cell will start to divide into 2 cells, then four, eight, sixteen and so on until 5 or 6 days later there are over 100 cells. This embryo is known as a blastocyst at this stage. There are two distinct areas seen in an expanded blastocyst; the inner cell mass - destined to become the foetus, and the outer cell layer called trophectoderm - destined to become placenta. For testing with PGS, a small number of cells (approximately 5) are removed from the trophectoderm. This is called a biopsy and this procedure is performed by a senior embryologist skilled in biopsy techniques. The biopsied cells are washed and placed in a small tube and sent for PGS analysis to our diagnostic laboratory. Studies have shown removal of cells from the trophectoderm layer is not detrimental to the further development of the embryo and thousands of babies have been born as a result of this procedure.

Blastocyst Biopsy



Biopsied embryos are immediately cryopreserved (frozen) using a method called vitrification. The results of the PGS analysis are available 2 weeks later. Euploid or normal embryos will be available for transfer in a frozen embryo transfer cycle at a later date, to be arranged with your consultant at your follow up appointment.

Advantages of Blastocyst Biopsy

- Several cells are removed leading to an accurate genetic analysis compared to single cell biopsy at an earlier stage of embryo development.
- Evidence suggests that biopsy at this stage may be safer for the continued development of the embryo as the removed cells have already differentiated into the trophectoderm layer (future placenta) rather than taking cells from the developing embryo itself at an earlier stage.
- Aneuploidies arising from both egg and sperm can be detected.
- Clinical pregnancy and live birth rates are markedly higher in frozen embryo transfer cycles in general and data are showing improved clinical and birth rates following PGS and transfer of euploid embryos in frozen embryo transfer cycles. [1, 2 & 3]

Disadvantages of Blastocyst Biopsy

- Approximately half of pre-implantation embryos reach the blastocyst stage. The percentage may be significantly less in women over the age of 39 years. This **can potentially reduce the number** of blastocysts available for biopsy, limiting in some cases the selection benefits of the analysis.
- Different cell lines (known as mosaicism) *may* be detected in the biopsied sample – see below for more information about mosaicism.

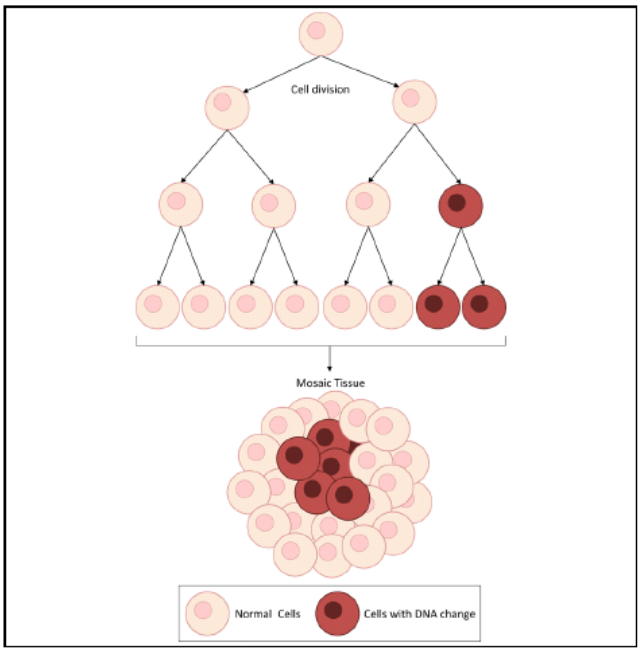
EMBRYO TESTING and PGS ANALYSIS

The biopsied cells are analysed using a technique called Next Generation Sequencing (NGS). This test analyses all 24 chromosomes to check for extra or missing copies; it is essentially a chromosome count. NGS works by detecting thousands of sequences of DNA along the length of each chromosome. The test is highly sensitive and accurate for detecting chromosome numbers.

Accuracy of NGS

The aim of PGS using NGS is to detect aneuploidy involving losses and gains of whole chromosomes. The majority of PGS cases (over 90%) produce an accurate aneuploid or euploid result. Due to the increased sensitivity of NGS it is also possible to detect relatively large deletions or duplications of sections of chromosomes. These are called partial segmental aneuploidies or PSAs. The clinical significance of embryos with missing or extra sections of chromosomes is currently unknown and these embryos are considered aneuploid.

Mosaicism and Mosaic Embryos



What is Mosaicism?

As cells divide a spontaneous genetic change can arise in a cell that continues to reproduce leading to two distinct cell lines in the tissue (see picture). This can happen in embryos that are undergoing fast cell division in the early stages of development. The presence of two cell lines is called mosaicism.

Because NGS is a highly sensitive test, the detection of mosaicism is now possible. Most embryos will either be screened as euploid or aneuploid. In a minority of PGS cases (<9%) mosaic cell lines may be detected. Mosaicism will usually involve whole chromosome losses and gains. If a mosaic cell line is detected in **the biopsied sample**, the laboratory will report the mosaic change and you will have a follow up with your consultant or a genetic counsellor to discuss the recommendations for embryo transfer.

Is it possible to know the sex of the embryo?

No. The HFEA Act prevents social sex selection in the UK and information regarding the sex of the embryo will not be available.

When are embryos transferred following PGS?

Euploid or normal embryos are transferred to the womb at a later date in a natural or stimulated frozen embryo replacement cycle. A follow up appointment with your consultant will be recommended once the results of the PGS testing are available to set up this phase of your treatment cycle.

Please Note: It is CARE policy to transfer ONE euploid embryo per cycle regardless of age.

BENEFITS OF PGS

Increased chance of becoming pregnant

It is well known that IVF pregnancy rates decrease dramatically with maternal age. Aneuploid embryos have much lower implantation rates than normal embryos. By performing PGS and transferring euploid or normal embryos, the latest data are showing a corresponding increase in pregnancy and birth rates. [4]

Fewer miscarriages

Approximately 35% of pregnancies are lost through miscarriage in women aged 35 and older. Aneuploidy is now considered to be the main cause for the majority of these pregnancy losses. By transferring euploid or normal embryos the number of miscarriages following PGS are significantly reduced.

Diagnostic information

Understanding the aneuploidy level in embryos can allow you to make informed decisions about your future fertility options.

Multiple Pregnancies

Transferring a single embryo reduces the risk of multiple births and complications arising from a multiple pregnancy. The chance that a single transferred blastocyst will divide and result in an identical twin pregnancy is very low (3%).

No significant health risks to children born following embryo biopsy

The first births following embryo biopsy for genetic screening were reported in 1990. Since then, thousands of children have been born and the health risks following embryo biopsy are no different than IVF/ICSI pregnancies in general.

RISKS OF PGS

All types of medical treatments and procedures have risk and your consultant will speak with you about these risks before you consent to PGS treatment in your IVF cycle. Below are some of the possible risks associated with PGS.

Embryo biopsy

The micromanipulation technique used for the biopsy has been performed on embryos for over 25 years. The risk of accidental damage to the embryo is extremely low (approximately 0.2%). If a blastocyst is damaged by the technique the embryo will stop growing and would not be suitable for transfer. Other routine procedures on embryos during IVF such as assisted hatching performed by making a small opening in the covering of the embryo have not been found to have any adverse effects on embryo development.

Effect of cryopreservation (freezing) on embryo development

Embryos can lose some of their cells following the vitrification and thaw procedure with no adverse effect demonstrated in babies born over the past 20 years from frozen embryos. An unanswered question is whether biopsied embryos implant less than ones that have not been biopsied. Data regarding this is incomplete. Potentially, embryo biopsy may lower implantation rates slightly while selection of chromosomally normal embryos using PGS may increase it. The beneficial effects of PGS outweigh the unconfirmed potential

lowered implantation rate and emerging data show that pregnancy rates are as good or higher, in frozen embryo transfer cycles compared to fresh cycles. [5]

LIMITATIONS OF PGS

No Result

The majority of embryos produce a result, however, in approximately 5% of cases the biopsied samples do not yield a result. If this happens we cannot tell if the cell(s) has a normal or abnormal number of chromosomes. There are two main reasons for 'no result'.

- The removed cell may not contain a nucleus (the structure in the cell containing the chromosomes). However, trophectoderm biopsies in blastocysts take several cells and so 'no result' due to lack of nucleus is less likely.
- DNA within the biopsied cells may degrade and the DNA amplification process is then unsuccessful leading to 'no intact DNA' which is equivalent to 'no result'.

In these circumstances it would still be possible to transfer the embryo with the understanding that all the potential benefits of PGS will not apply for the particular embryo transfer. The embryology team will discuss this situation with you, should it arise, and advise on the appropriateness of transfer or the possibility of re-biopsy of embryo(s).

Please note the following important points:

- CARE will only replace an embryo with 'no result' if there are **no euploid embryos** to replace.
- An unscreened or 'no result' embryo CANNOT be transferred with a euploid embryo at the same time.[6] Since it is CARE policy to transfer a single embryo, this situation will not arise.

Reduced Number of Embryos Available for Transfer following PGS

PGS may reduce the number of embryos available for transfer, as some or all will be identified as aneuploid. These embryos will be either retained for our QC/QA research and training or humanely discarded in accordance with your signed consent form. Please Note: **If you have selected 'embryo discard' we will not require you to sign a further consent form and your aneuploid embryos will removed from storage.**

Misdiagnosis

It is estimated this is approximately 5% may yield a misdiagnosis due to false positive or false negative results. For this reason CARE recommend prenatal diagnosis following PGS.

False positive result

Is where the biopsy analysis indicates that there are an abnormal number of chromosomes but the embryo is euploid. This may occur due to mosaicism and is estimated to be very low. Analysis of several cells obtained during blastocyst biopsies leads to a more accurate analysis with a % detection of mosaicism and data is incomplete on the false positive rate of NGS. There are unlikely to be adverse clinical consequences of this finding, as the embryo would not be transferred in this case.

False negative result

Is where the biopsy analysis suggests that the embryo is euploid or normal but actually the embryo is aneuploid. This is more significant because an embryo will be transferred with a euploid result. This may occur due to mosaicism. Historically, embryos were biopsied at the 8-cell stage of development (day3) and mosaicism was not detected in a single cell. Data are limited on the false negative result rate of NGS but is estimated to be less than 5%. Testing several cells obtained during blastocyst biopsies, the recommended strategy for PGS, leads to a more accurate analysis with the possible detection of mosaic cell lines.

Due to the low risk of misdiagnosis CARE recommend all patients who are pregnant after PGS should undergo prenatal diagnosis (see below).

Technology used to detect chromosome numbers
Next Generation Sequencing (NGS) can detect abnormalities involving the loss or gain of an entire set of chromosomes such as triploidy, the presence of 69 chromosomes; a very rare outcome. Large deletions and duplications can be detected of sections of chromosomes known as partial segmental aneuploidies; the clinical significance of these changes are currently unknown. The detection of small micro changes (deletions and/or duplications) along the length of the chromosomes is not possible using NGS.

CARE Recommendation
Because of the low possibility of misdiagnosis CARE recommend that all patients who become pregnant following PGS undergo prenatal diagnosis (see below). Please discuss this with your obstetrician during your pregnancy.

OTHER CONSIDERATIONS DURING PGS TREATMENT

1. Embryos may not develop to blastocyst. Embryo biopsy would not be performed but the unscreened embryos could be transferred. The benefits of PGS would not apply in this case.
2. Trophectoderm biopsy can only be performed on expanded, good quality blastocysts. If there are no suitable blastocysts to biopsy then unscreened early or lower quality blastocysts may be transferred but again the advantages of PGS would not apply.
3. The thaw survival rate of biopsied embryos is greater than 95% with the technique of vitrification, however, we cannot guarantee that all biopsied blastocysts will survive the thawing procedure and be available for transfer.
4. If all embryos are found to be aneuploid, embryo transfer would not be possible.
5. Technical issues
    a. Transporting Samples: The biopsied cells will be couriered to the diagnostic laboratory for PGS analysis. Although unlikely, it is possible that adverse circumstances may prevent the sample from arriving at its destination. In this case embryos could still be transferred but they would be unscreened and the benefits of PGS would not apply in this situation.
    b. DNA amplification failure resulting in 'No Result'. In this case embryos could still be transferred but they would be unscreened and the benefits of PGS would not apply in this situation

WHAT HAPPENS AFTER EMBRYO TRANSFER?

Pregnancy Test
You will have a pregnancy test 2 weeks after embryo transfer and we ask you to please contact the nursing team and let us know the outcome of your test.

Prenatal Diagnosis
Because of the low possibility of misdiagnosis we recommend that all patients who become pregnant following PGS undergo prenatal screening. Please discuss this with your obstetrician when you are pregnant. There are three (3) options available to confirm the chromosome status of any pregnancy achieved by IVF with PGS.

Please note in routine antenatal care the NHS will assess your risk of chromosome abnormalities based on maternal age and this will not take into account that you have had PGS. You may wish to discuss this further with your CARE consultant.

Non Invasive Prenatal Testing (NIPT)
This is a simple blood test from a pregnant mother taken at 10 weeks and combined with an ultrasound scan can determine whether the pregnancy is at low risk of the chromosome abnormality due to the presence of an extra chromosome 21 (Downs) 18 (Edward) or 13 (Patau) syndromes. The test can also detect aneuploidies arising from the sex chromosomes (X and Y).

Chorionic Villus Sampling (CVS)
Chorion Villus Sampling (CVS) sometimes referred to as Chorionic Villus Biopsy (CVB) can be performed at around 11 weeks of pregnancy when a small sample of cells can be taken from the placenta. This tissue derives from the outer layer of the embryo (trophectoderm) tested during PGS.

Amniocentesis
Amniocentesis is usually performed between 15 and 20 weeks of pregnancy. A small amount of fluid is taken from the amniotic sac surrounding the foetus. Cells from the foetus are isolated from this fluid and analysed for the presence of 46 chromosomes.

The exact test performed depends on the protocols used by your Obstetric Team in collaboration and discussion with you.

OTHER SERVICES

Follow- up PGS Program
We invite all our patients who achieve pregnancy after IVF with PGS to participate in our Follow up Program. Information regarding pregnancy, pregnancy outcome and child development will be requested. Please contact your nursing team at your CARE clinic.

Additional Support
Fertility treatment can be an emotionally challenging time for many patients. Further support is available to you throughout your proposed treatment; appointments can be made with our fertility counselling service available at all CARE clinics.

Genetic Counselling	is available – please contact your CARE clinic for more information.
Counselling Services	are available – please contact your CARE clinic.

Support Groups

Fertility Network UK	http://www.infertilitynetworkuk.com/
HFEA	http://www.hfea.gov.uk/fertility-treatment-help.html

Please contact your clinic for more information if you would like to know more about groups in your local area.

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NOTES: