Genome-Wide SNP Genotyping

As you may recall, in 2003 we reported the findings from our initial genome-wide scan of 254 PROGRESS families, which highlighted regions of several chromosomes that may contain gene(s) responsible for HPC. This scan used “microsatellite” markers, which are short sequences of between 1-4 repeating nucleotides or base-pairs (see Glossary of Terms).

However, the microsatellites are widely spaced across large regions of the chromosome, which can contain many genes. So, any significant “hits” discovered via linkage analysis require quite a lot of additional searching and narrowing of the region in question to find the actual disease-associated gene(s) and specific mutations in those genes.

As with all technology, the methods available for genotyping have advanced rapidly. It is now possible to look for genome-wide changes in DNA in much smaller segments of the genome by genotyping “Single Nucleotide Polymorphisms” or SNPs. SNPs represent a change in a single nucleotide at a particular location in the genome between individuals, e.g., the change in the DNA segment AAGGTTA to ATGGTTA on a particular chromosome where the second “A” in the first segment is replaced with a “T”. Genome-wide SNPs are much denser than microsatellite markers, occurring closer together and therefore allowing for much finer mapping of the genome. This higher “density” means that SNPs provide more power to detect linkage.

However, genome-wide SNP genotyping is quite costly. Fortunately, we submitted a proposal to the Center for Inherited Disease Research (CIDR) that was approved, and they are currently completing a genome-wide SNP linkage scan using 2,155 DNA samples from all 307 PROGRESS families. The DNA samples were shipped to CIDR in June and we expect to receive the genotyping results soon. As always, once we have these data analyzed we will keep you updated on results via our next newsletter.

GLOSSARY OF TERMS

Nucleotide:
These are the basic building blocks of DNA, and are made up of one of 4 possible chemical bases plus a sugar molecule plus a phosphate backbone. The 4 possible chemical bases are: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T). Nucleotides join together to form long strands of DNA. The particular sequence of nucleotides on each DNA strand determines the genetic code of each individual person.

Base-Pair:
Nucleotides are attracted and held together by a hydrogen bond to form base-pairs. Adenine always bonds with Thymine and Cytosine always bonds with Guanine.
Past and Present Analyses

In the last newsletter, we mentioned an interesting association between a region on the long arm of chromosome 22 and more aggressive forms of prostate cancer. At least three papers have reported on this finding for chromosome 22q: 1) an analysis of 123 PROGRESS families with at least 2 men with aggressive prostate cancer; 2) an analysis of a subset of 269 families from the ICPCG (International Consortium for Prostate Cancer Genetics) dataset with 5 or more affected men with aggressive disease features; and 3) a recent analysis of a subset of the ICPCG families with evidence of linkage to this region on chromosome 22q, containing at least 4 men affected with prostate cancer.

The latter analysis further narrowed the identified region to 22q12.3, which contains over a dozen potential “candidate” genes. We and others are now genotyping additional markers in this region with the aim of further narrowing the region and identifying the actual gene mutation that may contribute to the development and progression of the disease.

A second analysis of 166 ICPCG families (including some PROGRESS families) with at least three men with features of more aggressive prostate cancer observed suggestive linkage for chromosomes 6p, 11q, and 20p (Schaid et al., Human Genetics 2006; 120: 471). When the families were grouped by average age at diagnosis, the signals on chromosomes 6 and 20 increased for families with an average age at diagnosis greater than 65 years, while the finding on chromosome 11 was strongest for families with an average age at diagnosis of 65 years or younger. The findings on all three of these chromosomes have been corroborated by other studies.

In the analysis of 123 PROGRESS families mentioned above, there was a suggestive linkage signal on chromosome 6 for families with an average age of diagnosis less than or equal to 58 years and a suggestive linkage signal on chromosome 20 for families with no evidence of male-to-male transmission (Stanford et al., The Prostate 2006; 66: 317). In the earlier genome-wide scan of 1,233 ICPCG families, a suggestive linkage signal was found on chromosome 11q for 606 families with an average age at diagnosis of 65 years or younger (Xu et al., Am J Hum Genet 2005; 77: 219).

The general message from these and other results is that there are multiple genetic loci associated with the clinical features of more aggressive forms of prostate cancer, which will provide useful avenues for ongoing and future research into the genetic composition of this disease.

Follow-Up Survey Update

As promised in the last newsletter, we mailed our second follow-up survey to all PROGRESS participants starting this past summer. From the initial mailing of 1,663 surveys, we received 1,072 (64%) returned surveys, 980 of which were completed. Participants who did not return their survey after a period of six weeks were given a reminder call, which has proved to be very helpful.

Of the 591 participants we have called, 155 have returned their survey in the mail or completed the surveys by phone, bringing the grand total of completed surveys to 1,227 or 74%. Obviously, we would like to improve on this percentage, so if you have requested a second survey or have stated that you still have your survey and will return it in the near future, we would encourage you to do so as soon as possible.

As with the first follow-up survey, an important aim of this survey is to update our database with any new prostate cancer diagnoses and reports of other cancer diagnoses in family members. We would like to thank all of you who have already returned your survey and helped us to make sure we have the most accurate and current information possible. Your cooperation is crucial to our continuing efforts to understand hereditary prostate cancer. We hope you liked the blue pin, which we sent as a token of our heartfelt appreciation.
We previously identified a region of significant linkage on chromosome 7q11-21 in our subset of 18 Jewish families. In order to identify the sequence or order of base-pairs that may be shared by all the men with prostate cancer in these families, and which might represent the original “founder” sequence in this population, we genotyped 4,852 genetic markers or single nucleotide polymorphisms (SNPs) in this region.

The most significant results were in a region that contains 16 known genes. Ongoing research efforts are now concentrating on this much smaller region on the long arm of chromosome 7, with the hope of identifying the gene responsible for increasing the susceptibility to hereditary prostate cancer in these families.

A link between kidney cancer and prostate cancer has also been shown in several studies. Men with a type of kidney cancer known as “papillary renal cell carcinoma” were found to have a higher risk of developing prostate cancer, while first-degree relatives of patients with kidney cancer have a threefold increased risk of developing prostate cancer compared to people with no family history of kidney cancer. A linkage analysis of 15 PROGRESS families with a member with primary kidney cancer initially showed two regions of suggestive linkage on chromosomes 11q and 4q (Johannesson et al., The Prostate 2007; 67: 732). When two families with a rarer form of kidney cancer, transitional cell carcinoma, were removed from the analysis, the LOD score for chromosome 11q became significant, reaching 3.2. This result supports our approach of grouping HPC families according to the presence of other cancers. The identified region on chromosome 11q contains several interesting genes, which will require further follow-up.

The initial process of identifying families who reported colon cancer in either the baseline or follow-up survey has begun and once we have confirmed the colon cancer cases, a linkage analysis will be carried out in this subset of PROGRESS families. As usual, we will continue to send you updates on our findings as soon as they are available.

There are two well-established genes that increase the risk of developing breast and ovarian cancer known as BRCA1 and BRCA2. The BRCA2 gene also has been the focus of a significant amount of research related to hereditary prostate cancer (HPC) for several reasons: 1) some studies have shown that breast, ovarian and prostate cancer tend to co-occur in families; and 2) studies have shown an increased risk of prostate cancer in the male members of families known to carry BRCA2 mutations, suggesting that the same mutation may be involved in the development of both types of cancer. However, few studies have looked at the occurrence of BRCA2 mutations in hereditary prostate cancer (HPC) families.

We recently conducted an analysis of BRCA2 mutations in a subset of 194 PROGRESS families, including some with members with breast cancer, ovarian cancer, pancreatic cancer, with Jewish ancestry, or with early onset prostate cancer. A total of 266 individuals were screened, however none showed evidence of deleterious mutations within the BRCA2 gene (Agalliu et al., Clin Cancer Res 2007; 13: 839). Two smaller studies similarly did not find any disease-associated BRCA2 mutations in affected members of HPC families, which suggests that mutations in the BRCA2 gene do not play a major role in familial prostate cancer.
As you may know, the PROGRESS study is one of about a dozen members of the International Consortium for Prostate Cancer Genetics (ICPCG), which is conducting meta-analyses of hereditary prostate cancer families from around the world.

In our last newsletter we described our participation to date, which has only involved sending family-level data to a centralized Data Coordinating Center to be used in combined linkage analyses. The grant renewal for the ICPCG now requests that participating groups send individual-level genotypes and data in order to conduct more detailed analyses. Importantly, no individual identifiers will be included, and only unique study numbers will be used to ensure confidentiality. However, we felt, in conjunction with the advice of our Ethics Review Board, that it was necessary to obtain consent from PROGRESS study participants to submit their individual-level data to the ICPCG. Of the consent forms we sent out, 1,090 were returned, for an overall response level of 65%.

We would like to thank those of you who returned the consent form, letting us know if we can or cannot include your data. If any of you still have the consent form and do wish to be included, or would like us to send you another form for the same purpose, there is still time to do so. We will keep you up-to-date regarding these new analyses and the results as they become available.

At this time, the grand total of PROGRESS families is 307! We thought you might like to see where participating members are located.