The Children's Hospital *of* Philadelphia
Hope lives here.

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June 9, 2010

Kate Hettiger
Medical Chair
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P.O. Box 94
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Dear Kate:

It has been a year since my laboratory at The Children's Hospital of Philadelphia received the generous gift from PKS Kids in support of our ongoing research into the molecular causes of Pallister-Killian Syndrome. I wanted to take this opportunity to provide a brief report on the progress of our research since then.

As detailed in the attached report, our genomic studies have enabled us to narrow our research focus to a very specific region on chromosome 12p – even to the level of individual candidate genes – that we believe will impact our ability to diagnose and counsel families. Our recent findings are also laying the groundwork for understanding PKS on a very fundamental level, which we hope will lead to the development of better management and therapeutics for individuals with PKS in the future. These studies have provided the preliminary data needed to prepare a grant application to the NIH for a more comprehensive PKS research project, which we plan to do within the coming year.

This initial progress in understanding the molecular basis of PKS was made possible through the generosity of PKS Kids and the dedicated families who have supported our studies. I am honored to be the first recipient of a research grant from your outstanding organization. If there is an opportunity for additional support from PKS Kids to help further advance our research here at CHOP, I would be happy to provide you with more information on how we envision that a new grant could help forward this cause.

Thank you for the ongoing support and the incredible work you and PKS Kids are doing for children and families. Please feel free to contact me (as always!) with any questions.

Sincerely,

Ian Krantz, MD

Applying Novel Genomic Tools Towards Understanding Pallister-Killian Syndrome

A Report to PKS Kids

Ian Krantz, M.D.
Division of Human Genetics, The Children's Hospital of Philadelphia

Pallister-Killian Syndrome (PKS) is a multisystem developmental disorder caused by tetrasomy 12p (4 copies of the short arm of chromosome 12, also referred to as an "isochromosome 12p") that exhibits tissue-limited mosaicism. The fact that not all cells in an individual with PKS have the extra chromosome 12p material (called mosaicism) is an important feature of PKS, and makes it important to test the right tissue in order to confirm a diagnosis (e.g. the extra isochromosome 12p is often lost from the blood of older children, necessitating testing from skin, or other tissues, to confirm a diagnosis). Some individuals with PKS have interstitial duplications (resulting in 3 copies -- instead of the typical 4 -- of the short arm of chromosome 12p) some of which are only partial copies of the short arm.

The spectrum of clinical manifestations in PKS, while very characteristic, can be wide and includes craniofacial dysmorphia, clefts, ophthalmologic, audiologic, cardiac, musculskeletal, diaphragmatic, gastrointestinal, gentinourinary, and cutaneous differences in association with cognitive impairment and seizures. Growth parameters are often normal to elevated at birth with deceleration of growth postnatally. The prevalence of PKS has been estimated be ~1/20,000 live births but is likely under-ascertained since tetrasomy 12p is often not present in the blood and requires fibroblast (skin) or other tissue sampling to identify.

Through our work here at The Children's Hospital of Philadelphia (CHOP) and in collaboration with PKS Kids, we have been able to identify and clinically follow over 45 children with PKS and, thanks to gracious start-up funds made available through PKS Kids, we have initiated genomic analyses to better understand the manner in which additional copies of chromosomes 12p result in the highly specific features seen in PKS.

While the cause of PKS has been well established for many years (extra copies of the short arm of chromosome 12), how these extra copies result in the clinical findings seen in PKS remains unknown. By identifying the critical genetic factors, on chromosome 12, or elsewhere in the genome ("genome" refers to all of the DNA material and genes housed on all of the two sets of 23 chromosomes in every cell of our body), we will begin to understand the important elements that cause the individual features seen in PKS and this will be the start of a process towards identification of improved therapeutics for affected kids and adults.

We have implemented several state-of-the-art genomic approaches to better characterize and understand what is happening in the cells of individuals with PKS. Using extremely high resolution DNA arrays called single nucleotide polymorphism (SNP) arrays with over 600,000 bits of genetic information representing the whole genome, we have been able to accurately quantify degrees of mosaicism (and loss of the isochromosome) from skin and blood, and to determine parent of origin and to pinpoint exactly at what stage of cell division (called "meiosis") of the egg or sperm the extra chromosome developed. These tests also are proving to be much more accurate in identifying the extra 12p chromosomal material from

the blood of older individuals with PKS, making testing from the skin a less commonly needed test.

We have also used these arrays to define a region on chromosome 12p that contains the critical genes necessary for the clinical features in PKS. This region is called the "PKS critical region" and contains approximately 30 genes. This narrows down our search for the actual causative genes on the short arm of Chromosome 12 from around 300 to 30. We believe that only a few genes, or even only one gene, on the short arm of chromosome 12p is responsible for the features we see in PKS; identifying the actual genes that have an effect when present in 3 or 4 copies will be critical to understanding what is actually causing the clinical difference in PKS.

In parallel to these studies we have performed genome-wide expression array analysis (using Affymetrix HG-U133 plus 2.0 GeneChip arrays) on skin cells (called "fibroblasts") from 17 individuals with PKS. These studies allow us to see what genes in these cells are turned "on" (or "up") or "off" (or "down") (called "expression" or how the genes are "expressed") in a different way from cells from individuals without PKS. Since what we think is happening in PKS is that a few critical genes on chromosome 12p, being present in extra copies, are talking to other genes in the genome (on other chromosomes) and telling them to be turned "on" or "off" at an inappropriate level, which in turn causes problems in development. By identifying these downstream genes we hope to get a complete picture into what is actually causing the individual features, seen in constellation, in PKS.

Through this understanding we hope to identify targets (genes or proteins) that we can develop therapeutics against to help kids with PKS. From these studies, 354 genes (out of the 23,000 genes in our genome) were identified that were dysregulated with statistical significance (180 up-regulated and 174 down-regulated). The most statistically-significant dysregulated genes on 12p mapped to within the SNP-array defined critical region on 12p13, as described above. Several of the dysregulated genes identified are excellent candidates for causing some of the features seen in PKS and more work is needed to confirm these findings.

This research has taken us from a very low-resolution understanding of what causes PKS to an extremely high-resolution view, down to a specific critical region and even individual genes, that are critically important to this diagnosis. The knowledge learned even in this short time will impact our ability to diagnose and counsel families and has laid the groundwork for understanding PKS on a very fundamental level that will hopefully lead to improved management and therapeutics for individuals with PKS.

This research could not have even been contemplated without the amazing help and support of the families of children and adults with PKS, the PKS individuals, and the visionary support of the PKS Kids Foundation, all of whom understand the importance that this type of research will have on the future of PKS children and families. The preliminary results obtained through these studies will be used to support a grant application to the National Institutes of Health (NIH) to further support this important work.