

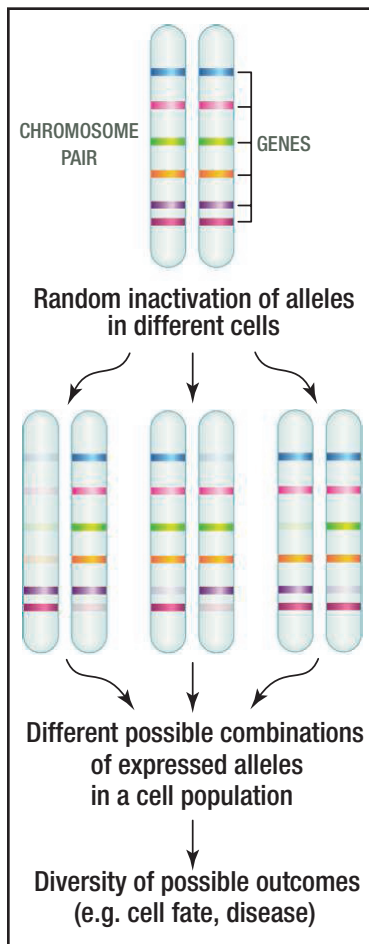
with genes reside. Conversion of this RNA to double-stranded cDNA and analysis on a SNP array generated “transcriptosome-derived genotypes” that allowed monoallelic expression to be identified. Filters were used to minimize cDNA genotyping artifacts. About 10% of SNPs were reliably called from this analysis, which was expected since most of the other SNPs are likely present in regions of the genome that are not expressed by B cell lines that were studied.

As proof-of-concept, the investigators first showed that random inactivation of X-chromosome genes could be detected in the clonal cell lines and then demonstrated as an example of their approach that monoallelic expression of the amyloid precursor protein gene could be detected. They next turned to genome-wide screening.

On the array used for analysis, there were SNPs present for ~11,000 genes. They were able to detect allele-specific transcription for ~4,000 genes in 2 or more cell clones. Of the ~4,000 genes examined, 2.2% were detected as monoallelically expressed with multiple informative SNPs per gene per clone. An additional 7.3% of assessed genes were identified as monoallelically expressed based on a single informative SNP per gene per clone. The genes included both B cell-specific genes and ubiquitously expressed genes. The investigators suggested a conservative estimate that over 1,000 genes are subject to random monoallelic expression in humans.

Several interesting observations were made. For example, the choice of expressed allele was made independently for each gene within a given clonal cell line. This is in contrast to the chromosomal-wide coordination characteristic of X-inactivation. Another finding was that a disproportionately large fraction of genes coding for cell surface proteins—transmembrane receptors and surface proteins was detected.

The authors concluded by suggesting that at least 1,000 human genes display random monoallelic transcription



Generating diversity. Alleles are randomly inactivated on a pair of chromosomes in a human somatic cell. The various patterns of inactivation in progeny cells are then stabilized (epigenetically). This can generate diverse cellular and physiological outcomes. Reprinted with permission Ohlsson R. Science. 2007;318:1077-8. Copyright © 2007 AAAS. All rights reserved.

that could contribute to genetic diversity within tissues of an individual as well as between individuals. A commentary by Ohlsson¹ notes that although monoallelic expression has been known in humans, this study by Gimelbrant expands the concept further especially by documenting it in a much larger number of genes than previously appreciated. He briefly discusses possible mechanisms that could account for the phenomenon as well as its potential role in modulating disease (Figure).

Gimelbrant A, Hutchinson JN, Thomson BR, Chess A. Widespread monoallelic expression of human autosomes. Science. 2007;318:1136-40.

Editor's Comment: This is one of several publications in recent years that challenges what we were taught about mendelian genetics. Of note, several genes relevant to human growth disorders were identified as displaying monoallelic expression including the growth hormone receptor gene (*GHR*) and genes that harbor mutations responsible for *Ellis van Creveld syndrome (EVC)* and the *trichorhinophalangeal syndrome 1 (TRPS1)*. It seems quite plausible that monoallelic expression of these genes could contribute to the clinical variability of these conditions.

Lymphoblastoid cells have very different functions compared to chondrocytes, osteoblasts and other cells that contribute to skeletal growth; and their patterns of gene expression may differ dramatically. Screening the latter cells for monoallelic transcription would be technically much more difficult than for lymphoblastoid cells, but it would likely reveal monoallelic expression of additional growth related genes.

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Reference

1. Ohlsson R. Science. 2007;318:1077-8.

Stroke, Cardiac Disease and Diabetes Mellitus in Hypopituitarism

The impact of long-term growth hormone deficiency (GHD) and of long-term growth hormone (GH) treatment on cerebrovascular and cardiovascular diseases and diabetes mellitus is unknown. Holmer et al evaluated

the incidence of nonfatal stroke and cardiac events and the prevalence of type 2 diabetes mellitus (T2DM) in a cohort of GHD patients and healthy controls. The authors also studied the effects of cardioprotective drugs and 6

years of GH-replacement treatment in this population. The incidence of nonfatal stroke and cardiac events was estimated retrospectively from questionnaires in 750 GHD patients (53% males and 47% females) and in 2314 matched population controls. GHD patients were recruited from the departments of endocrinology at all Swedish University hospitals and one county hospital. All patients were diagnosed as having severe GHD by dynamic testing (peak GH <3 mcg/L). The lifelong incidence of nonfatal stroke was tripled in GHD women and doubled in GHD men. A decline was noted in both genders following the detection of the first pituitary hormone deficiency and GHD, a period of time during which most patients received GH therapy. The lifelong incidence of nonfatal cardiac events declined in GHD men; GHD women had a higher prevalence of T2DM. Women were twice as likely to be taking lipid-lowering drugs as the population controls, while GHD men had a 28% higher prevalence for the use of antihypertensive medication. The authors concluded that the decreased risk of nonfatal stroke in both genders and of nonfatal cardiac events in GHD men may be due to the larger prescription of cardioprotective drugs and to 6 years of GH-replacement. The increased prevalence of T2DM in GHD women can be partly attributed to a higher body mass and to decreased physical activity.

Holmer H, Svensson J, Rylander L, et al. Nonfatal stroke, cardiac disease and diabetes mellitus in hypopituitary patients on hormone replacement including growth hormone. *J Clin Endocrinol Metab.* 2007;92:3560-7.

Editor's Comment: *An increased incidence of cerebrovascular and cardiovascular mortality in patients with hypopituitarism on conventional hormone treatment, but without GH therapy, has been reported in recent epidemiological studies.^{1,2} GHD is believed to be responsible for the early atherogenesis in hypopituitarism,*

as cardiovascular risk factors have been improved with GH treatment in this group of patients. Glucose intolerance, T2DM, and hypertension are increased in GHD.³ Diastolic blood pressure tends to decrease with GH treatment, while insulin sensitivity is impaired following initial GH replacement, but may improve later as fat mass is reduced. This study showed that hypopituitary patients had a higher lifelong incidence of nonfatal stroke (triple in GHD women and double in GHD men), although cerebrovascular events decreased in men and women during the periods following the diagnosis and the treatment of the pituitary hormone deficiencies and of GHD. This decline was probably due to the long-term use of GH and the replacement of thyroxine and glucocorticoids. Additionally, patients may also benefit from the increased administration of lipid-lowering and antihypertensive medications. The increased prevalence of T2DM in GHD women could not be attributed to overtreatment with GH as the IGF-I level was at mid range. Additionally, acromegaly and Cushing's disease were excluded in these patients, thus the increased prevalence of T2DM was partly attributed to their higher BMI and their lower physical activity. Long-term surveillance for cardiovascular disease and T2DM seems necessary in hypopituitary patients; the institution of appropriate treatment with hormone replacement and cardioprotective drugs plays a positive role in decreasing the risk of nonfatal stroke and the risk of nonfatal cardiac events in men; an increased prevalence of T2DM seems to be present in GHD women.

Roberto Lanes, MD

References

1. Rosen T, Bengtsson BA. *Lancet.* 1990;336:285-8.
2. Bulow B, Hagmar L, Mikoczy Z, et al. *Lancet.* 2001;357:425-31.
3. Johannsson JO, Fowellin J, Landin K, et al. *Metabolism.* 1995;44:1126-9.

Growth and Metabolism in In Vitro Fertilization Children

In vitro fertilization (IVF) singleton children have an increased risk of malformations and low birth weight. They also face an increased risk of disorders with overgrowth partly due to abnormal methylation patterns of imprinted genes. Nutritional manipulation early in fetal life has also been shown to reduce methylation and over expression of non imprinted genes. Miles et al conducted a study regarding the long-term outcome of IVF children, an area in which there is still a lack of information. The authors investigated growth and changes in the metabolic and hormonal profile of this population. Healthy prepubertal children aged 4 to 10 years, born at term, after singleton pregnancy, were recruited into IVF and control groups. All subjects had been breastfed. There were 69 IVF children (5.9 years) and 71 control children (6.9 years). Anthropometric measurements and BMI were recorded, focusing on fat and glucose metabolism, and insulin-like

growth factor (IGF)-I levels. Both groups were matched for parental anthropometry, socio-economic factors and dietary conditions. IVF children were taller than controls (and girls were even more so) when height was corrected for parental height. This increase in stature was proportionate. It occurred despite a lower birth weight. The corrected BMI was lower in the IVF group and there was no difference in percent fat assessed by DEXA. There was a trend toward higher IGF-I levels in the IVF group with patients above 7 years of age having the highest levels. The IGF-I/IGF binding protein (IGFBP)-3 ratio was also increased. IGF-II was elevated as well in the IVF group without any age related effect.

For all children there was an association between tall stature and high IGF levels. A favorable metabolic profile was found in the IVF group with higher HDL, lower triglycerides and a low total to HDL/cholesterol