Intramural papers of the month

By Tara Ann Cartwright, Deacqunita Diggs, Ernie Hood, Deepa Singh, and Annah Wyss

- NTP develops novel testing strategy
- Solvent exposure before first birth may increase breast cancer risk
- GWAS meta-analysis reveals new DNA loci of lung function
- dGTP starvation has detrimental effects on cells
- Nrf2 increases ABC transporter expression at blood-brain barriers

NTP develops novel testing strategy

Researchers at the National Toxicology Program (NTP) have developed a new study design, the modified one generation (MOG) reproduction study, which was compared and contrasted with the new Organisation for Economic Co-operation and Development (OECD) 443 test guideline, the extended one generation reproduction study. The MOG study increases the detection of reproductive and developmental effects, by retaining more offspring for longer periods of time and with continued toxicity testing following gestational exposure to toxic agents. This method will reduce animal use in toxicity studies while capitalizing on toxicity endpoints.

In the MOG study, toxicant exposure occurs during gestation. Offspring are saved longer and under continued exposure conditions. This new design allows assessment of target organ toxicity, developmental toxicity, and clinical pathology; covers observations conducted during teratology studies; and allows analysis of fertility and fecundity. The advantages of the MOG study over the OECD 443 test guideline include a focus on the critical periods of the F_1 generation reproductive life cycle, measurements of reproductive structure and function in the same animals, and the incorporation of other developmental outcome data.

The MOG study can effectively utilize and reduce animal use versus conducting individual developmental and reproductive toxicity or 90-day toxicity studies. This method allows for a range of toxicity and teratology effects to be assessed from gestation to death. **(DD)**

Citation: Foster PM.
(http://www.ncbi.nlm.nih.gov/pubmed/24862797)
Regulatory forum opinion piece: new testing paradigms for reproductive and developmental toxicity - the NTP Modified One Generation Study and OECD 443. Toxicol Pathol; doi:10.1177/0192623314534920 [Online 26 May 2014].

Solvent exposure before first birth may increase breast cancer risk

In a study recently published in the journal Cancer Research, NIEHS scientists reported that women exposed to solvents before the birth of their first child may have an increased risk for breast cancer, particularly estrogen receptor-positive breast cancers. These findings suggest that the time between puberty and first birth, a crucial period of breast development, may be an important window of susceptibility.

To assess the association between lifetime occupational solvent exposure and breast cancer, the researchers used data from the Sister Study, a prospective cohort study of initially breast cancer-free women who have a sister previously diagnosed with breast cancer. In addition to evaluating how the timing of solvent exposure affects breast cancer risk, the investigators also studied the impact of solvent use within specific occupations. Women who worked with solvents as clinical laboratory technicians, maids and housekeepers, or production workers appeared to have an increased risk of estrogen receptor-positive breast cancer, though occupation-specific estimates were sometimes based on only a small number of breast cancer cases.

Although this study highlights both time periods and occupations in relation to solvent exposure and breast cancer risk, the authors say that additional large-scale studies are needed to further examine the association, by focusing on types of solvents. (See story.) **(AW)**

Citation: Ekenga CC, Parks CG, D'Aloisio AA, DeRoo LA, Sandler DP.
(http://www.ncbi.nlm.nih.gov/pubmed/24879566)
2014. Breast cancer risk after occupational solvent exposure: the influence of timing and setting. Cancer Res 74(11):3076-3083.

GWAS meta-analysis reveals new DNA loci of lung function

NIEHS researchers and their collaborators in a global consortium identified six new loci -specific regions of the genetic code - associated with individual variations in a widely used clinical measure of lung function. The genetic variants appear to influence an individual's forced vital capacity (FVC), a measure used to diagnose and monitor lung diseases. The new loci may shed light on mechanisms involved in lung development and the pathogenesis of lung diseases.

Using a large-scale meta-analysis approach, members of the research group analyzed the results of genome-wide association studies (GWAS) in 52,253 individuals from 26 countries. They followed up the FVC associations in 32,917 additional individuals of European descent. The analysis identified six new regions associated with FVC that had not been seen in previous GWAS lung function studies. The newly implicated regions were also followed up in samples from African-American, Korean, Chinese, and Hispanic individuals.

The genes closest to the six loci associated with FVC variation were expressed in lung tissue and in primary lung cells. The expression associations were confirmed in 762 whole blood samples, using expression quantitative trait locus analysis. Better understanding of the role genes play in normal lung development and pathogenesis may lead to identification of novel therapeutic targets for lung diseases. (See story.) **(EH)**

Citation: Loth DW, Artigas MS, Gharib SA, Wain LV, Franceschini N, Koch B, Pottinger TD, Smith AV, Duan Q, Oldmeadow C, Lee MK, Strachan DP, James AL, Huffman JE, Vitart V, Ramasamy A, Wareham NJ, Kaprio J, Wang XQ, Trochet H, Kahonen M, Flexeder C, Albrecht E, Lopez LM, de Jong K, Thyagarajan B, Alves AC, Enroth S, Omenaas E, Joshi PK, Fall T, Vinuela A, Launer LJ, Loehr LR, Fornage M, Li G, Wilk JB, Tang W, Manichaikul A, Lahousse L, Harris TB, North KE, Rudnicka AR, Hui J, Gu X, Lumley T, Wright AF, Hastie ND, Campbell S, Kumar R, Pin I, Scott RA, Pietilainen KH, Surakka I, Liu Y, Holliday EG, Schulz H, Heinrich J, Davies G, Vonk JM, Wojczynski M, Pouta A, Johansson A, Wild SH, Ingelsson E, Rivadeneira F,

Volzke H, Hysi PG, Eiriksdottir G, Morrison AC, Rotter JI, Gao W, Postma DS, White WB, Rich SS, Hofman A, Aspelund T, Couper D, Smith LJ, Psaty BM, Lohman K, Burchard EG, Uitterlinden AG, Garcia M, Joubert BR, McArdle WL, Musk AB, Hansel N, Heckbert SR, Zgaga L, van Meurs JB, Navarro P, Rudan I, Oh YM, Redline S, Jarvis DL, Zhao JH, Rantanen T, O'Connor GT, Ripatti S, Scott RJ, Karrasch S, Grallert H, Gaddis NC, Starr JM, Wijmenga C, Minster RL, Lederer DJ, Pekkanen J, Gyllensten U, Campbell H, Morris AP, Glaser S, Hammond CJ, Burkart KM, Beilby J, Kritchevsky SB, Gudnason V, Hancock DB, Williams OD, Polasek O, Zemunik T, Kolcic I, Petrini MF, Wjst M, Kim WJ, Porteous DJ, Scotland G, Smith BH, Viljanen A, Heliovaara M, Attia JR, Sayers I, Hampel R, Gieger C, Deary IJ, Boezen HM, Newman A, Jarvelin MR, Wilson JF, Lind L, Stricker BH, Teumer A, Spector TD, Melen E, Peters MJ, Lange LA, Barr RG, Bracke KR, Verhamme FM, Sung J, Hiemstra PS, Cassano PA, Sood A, Hayward C, Dupuis J, Hall IP, Brusselle GG, Tobin MD, London SJ.
(http://www.ncbi.nlm.nih.gov/pubmed/24929828)
2014. Genome-wide association analysis identifies six new loci associated with forced vital capacity. Nat Genet 46(7):669-677.

dGTP starvation has detrimental effects on cells

Researchers from NIEHS have shown, for the first time, that starvation of cells for 2'-deoxyguanosine 5'-triphosphate (dGTP), one of the four building blocks of DNA, can exert replication stress, ultimately leading to growth impairment and cell death. This adverse effect is similar to a phenomenon called thymineless death (TLD), cell death as the result of a depletion of another DNA precursor, 2'-deoxythymidine 5'-triphosphate (dTTP). The work suggests that targeting cells for death by dGTP starvation, as happens with TLD, may be a potential avenue for antimicrobial and anticancer therapies.

To study the effect of dGTP starvation, the scientists employed an *Escherichia coli (E. coli)* strain, which combined an allele of the dgt gene with a defective gpt gene. The dgt gene encodes an enzyme that hydrolyzes dGTP into deoxyguanosine and triphosphate, while gpt encodes an enzyme that converts guanine into guanosine monophosphate, both required for nucleotide pool maintenance.

This particular *E. coli* strain showed tenfold lower levels of dGTP, which led to replication fork collisions, double-strand breaks, and generation of lethal multibranched nucleoids. dGTP deprivation also led to DNA damage, as reflected by the induction of the SOS response, a set of events that result in cell cycle arrest and the induction of DNA repair. **(DS)**

Citation: Itsko M, Schaaper RM.
(http://www.ncbi.nlm.nih.gov/pubmed/24810600)
2014. dGTP starvation in Escherichia coli provides new insights into the thymineless-death phenomenon. PLoS Genet 10(5):e1004310.

Nrf2 increases ABC transporter expression at blood-brain barriers

Researchers from the NIEHS Laboratory of Toxicology and Pharmacology have defined the mechanism by which nuclear factor E2-related factor-2 (Nrf2) increases activity and expression of three ABC transporters at the blood-brain and blood-spinal cord barriers. These transporters are xenobiotic efflux pumps that prevent foreign chemicals, including therapeutic drugs, from entering the central nervous system (CNS), thereby limiting our ability to treat CNS disorders.

Previous studies indicated Nrf2, a redox-sensor and ligand-activated transcription factor that plays a critical role in cellular defenses against oxidative stress, was neuroprotective in stroke and traumatic brain injury. The study, published in the Journal of Neuroscience, showed that dosing rats with sulforaphane, an Nrf2 activator, increased P-glycoprotein, multidrug resistance-associated protein-2, breast cancer resistance protein expression at the blood-brain barrier, and reduced drug entry into the brain. In isolated brain and spinal cord capillaries, Nrf2 induction of ABC transporter expression and activity required tumor suppressor p53, p38 MAPK signaling, and the transcription factor, NF-kappaB. These experiments define a sequence of events in two brain barriers where Nrf2 signals through p53, p38 MAPK, and NF-kappaB to increase ABC transporter expression and activity.

These findings suggest caution when activating Nrf2 for neuroprotection, since increased drug efflux transporter expression would impair subsequent CNS pharmacotherapy. **(TAC)**

Citation: Wang X, Campos CR, Peart JC, Smith LK, Boni JL, Cannon RE, Miller DS.
(http://www.ncbi.nlm.nih.gov/pubmed/24948812)
2014. Nrf2 upregulates ATP binding cassette transporter expression and activity at the blood-brain and blood-spinal cord barriers. J Neurosci 34(25):8585-8593.

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