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The Widespread Threat of Calcium Decline in Fresh Waters

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Calcium concentrations are now commonly declining in softwater boreal lakes. Although the mechanisms leading to these declines are generally well known, the consequences for the aquatic biota have not yet been reported. By examining crustacean zooplankton remains preserved in lake sediment cores, we document near extirpations of calcium-rich *Daphnia* species, which are keystone herbivores in pelagic food webs, concurrent with declining lake-water calcium. A large proportion (62%, 47 to 81% by region) of the Canadian Shield lakes we examined has a calcium concentration approaching or below the threshold at which laboratory *Daphnia* populations suffer reduced survival and fecundity. The ecological impacts of environmental calcium loss are likely to be both widespread and pronounced.

Lake-water calcium concentrations are currently falling in softwater lakes in many boreal regions (*1–3*). Declining calcium is part of an expected concentration trajectory (*4*) that is linked to a reduction in the exchangeable calcium concentration of catchment soils (*5*). Although such reduction is part of the natural, long-term process of soil acidification, it is accelerated by other factors that vary regionally in importance [for example, acidic deposition (*1, 6*), reduction in atmospheric calcium inputs (*7*), calcium loss from forest biomass harvesting, and regrowth after multiple timber harvesting cycles (*2, 8*)]. Acidic deposition plays an especially complex role because it initially increases soil leaching of calcium and surface-water calcium concentrations. However, in watersheds with thin soils underlain by weathering resistant bedrock, the leaching rate typically exceeds the replenishment rate from weathering and atmospheric deposition (*9*) so that decades of acidic deposition result in the depletion of soil base saturation (*1, 6*). Depleted base saturation necessarily results in reduced calcium concentrations in runoff, and reduced acidic deposition further accentuates this trend (*7*). Surface-water calcium concentrations may even fall below pre-acidification levels (*4*). Watershed geochemists recognize the importance of soil calcium depletion as a factor
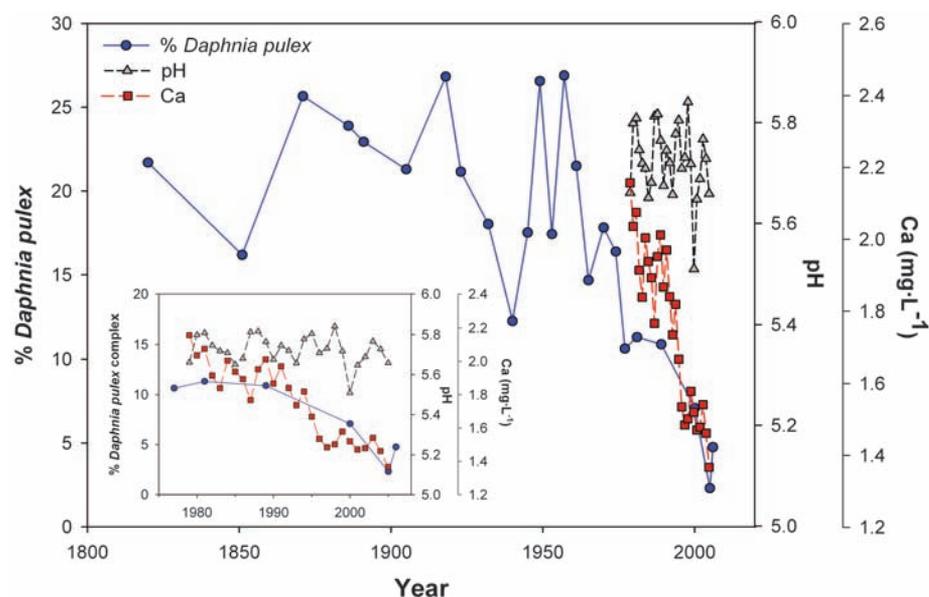
confounding the recovery of lakes from acidification (*1*).

Whereas the biogeochemical dynamics that underpin calcium decline are largely understood, little is known regarding the direct effects of declining calcium concentrations on the biota of softwater lakes, and clear evidence of such biological damage does not currently exist. We investigated the consequences of calcium decline for *Daphnia* in Precambrian Shield lakes that have aqueous calcium concentrations near or below 1.5 mg L^{-1}. This is the threshold concentration that has been experimentally demonstrated to reduce *Daphnia pulex* survival and to delay reproduction and reduce fecundity (*10*). First, using dated lake sediment cores, we examined temporal changes in calcium-rich

Daphnia species (keystone pelagic herbivores). Second, we used a regional paleolimnological survey to compare the abundances of modern-day and preindustrial daphniid subfossil remains in 43 lakes. Finally, to assess the current prevalence of the threat of aquatic calcium loss, we analyzed changes in pH and calcium in 770 lakes from the 1980s to the 2000s .

Although many calcium-rich organisms may be directly affected by declining aqueous calcium concentrations, we focused on the crustacean zooplankton because they leave identifiable remains of individuals in lake sediments that allow the reconstruction of detailed paleoecological records (*11*). Because they are important phytoplankton grazers and the principal prey of predatory macroinvertebrates and small, planktivorous fish in pelagic ecosystems, changes in the crustacean zooplankton community will profoundly influence aquatic ecosystems (*12, 13*). Partial life cycle bioassays with *D. pulex* (a species common to softwater lakes) indicate that calcium reductions below 1.5 mg L^{-1} delay reproduction and reduce clutch sizes, substantially reducing intrinsic rates of population growth (*10*). Furthermore, daphniids (Daphnidae, Anomopoda, Crustacea) have much higher calcium concentrations than other crustacean zooplankton taxa [2 to 8% of dry body weight compared with 0.2 to 0.4% for non-daphniid competitors (*14*)] and thus are probably more sensitive to calcium decline.

Because the stressors that have led to calcium decline pre-date water chemistry monitoring programs in most regions (*15*), a paleolimnological analysis of calcium-rich daphniid remains, using standard methods (*16*), was performed for Plastic Lake (45° 11′N, 78° 50′W)—a 32-ha dimictic, softwater [specific conductivity = 17 μS cm^{-1}

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Fig. 1. Changes in the relative abundance of remains from the *D. pulex* complex relative to other sedimentary cladoceran remains, ice-free whole-lake calcium, and the pH of Plastic Lake, Ontario, Canada. (Inset) Changes in these same three variables since 1976.

(where $1S = 1A/v$) 2000 to 2005 average] lake in south-central Ontario, Canada. Measured twice a month, the ice-free mean pH of the lake has been quite stable since the late 1970s at ~5.8 (Fig. 1), a value that has not changed much from a diatom-inferred (17) pH of 5.7 that pre-dates the onset of modern human influence (18). However, calcium concentrations have fallen from ~2.2 mg L^{-1} in 1980 to ~1.4 mg L^{-1} in 2006, with a period of steep decline occurring after 1991 (Fig. 1). Correlating with the decline in calcium, there has also been a decrease in the relative abundance of daphniid sedimentary remains (Pearson product moment correlation between percent of *Daphnia* and calcium = 0.95, $P < 0.05$) (Fig. 1).

Between 1985 and 2005, a mean calcium decline of 13% was observed in 36 lakes from the Muskoka region of the southern Canadian Shield in Ontario, concurrent with an increase in their average pH from 5.9 to 6.2 (Fig. 2A). During the 1980s, only 1 of the lakes had a calcium concentration <1.5 mg L^{-1}; now 5 of the 36 lakes are below this threshold. We compared recent sedimentary cladoceran remains and those deposited before European settlement (~1850) (19) from an additional set of 43 Muskoka lakes (including Plastic Lake). The relative abundances of all daphniids have decreased in 60% of the lakes having a present-day calcium concentration <1.5 mg L^{-1} and in 67% of the lakes with calcium concentration between 1.5 and <2.0 mg L^{-1} (Fig. 2B). These changes contrast with increases in *Daphnia* spp. relative abundances in all the lakes with calcium concentrations >2.5 mg L^{-1} (Fig. 2B).

We also identified long-term declines in the abundance of calcium-rich *Daphnia* spp. relative to increases in calcium-poor *Bosmina* spp. (14) in paleolimnological records from two other eastern North American lakes (Fig. 3). These lakes have calcium concentrations near 1.5 mg L^{-1} but have different acidification histories. Little Wiles Lake (44° 24′N, 64° 39′W; Nova Scotia, Canada; see inset map in Fig. 4) is naturally acidic (pH ~ 5.6) (20) and has experienced declining calcium concentration (21), whereas diatom profiles indicate that the pH remained stable (like Plastic Lake) during the period of maximum acid deposition in the 1970s (22). In contrast, Big Moose Lake (43° 49′N, 74° 51′W; New York, U.S.) has experienced a steady calcium decline throughout a period of acidification in the 1950s (to pH 4.6) (23) and a subsequent recovery in pH (current pH is >5.5) (24). Daphniid relative abundance dropped to trace levels in Little Wiles Lake during the mid-1970s (Fig. 3). Declines in daphniid populations were also detected in Big Moose Lake, and populations remain substantially below their pre-impact abundances despite a recovery of pH (Fig. 3). Hence, calcium decline may be preventing daphniid recovery from lake acidification in Big Moose Lake and perhaps other acidified lakes in eastern North America.

Concurrent changes in other limnological variables have often accompanied the regional aqueous calcium decline—notably an increase in pH (Fig. 4), a decrease in total phosphorus concentration (18, 25), and an increase in occurrences of predatory bass, *Micropterus* (26). The loss of daphniids in the low-calcium lakes cannot be attributed to these trends, which should favor the large, highly visible, calcium-rich daphniids
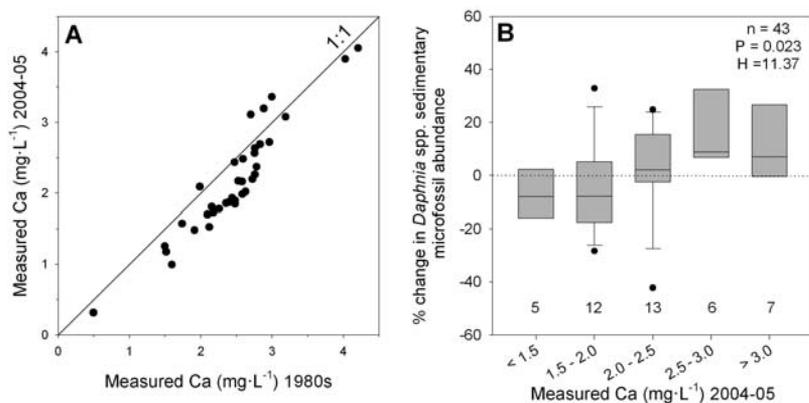
Fig. 2. Changes in ice-free season average lake-water calcium concentration and *Daphnia* spp. remains over time. (**A**) Changes in aqueous calcium from the 1980s to 2004 or 2005 of 36 softwater lakes from the Muskoka region of the Canadian Shield in Ontario (calcium declined in all but four lakes). (**B**) Change in the relative abundance of *Daphnia* spp. sedimentary remains since preindustrial times for 43 Muskoka lakes sorted by present-day calcium (Kruskal-Wallis nonparametric analysis of variance for all five classes, $P = 0.023$, $H = 11.37$, $df = 4$; inset values denote sample size within each calcium class). Error bars indicate the 10th (lower line) and 90th (upper line) percentiles.
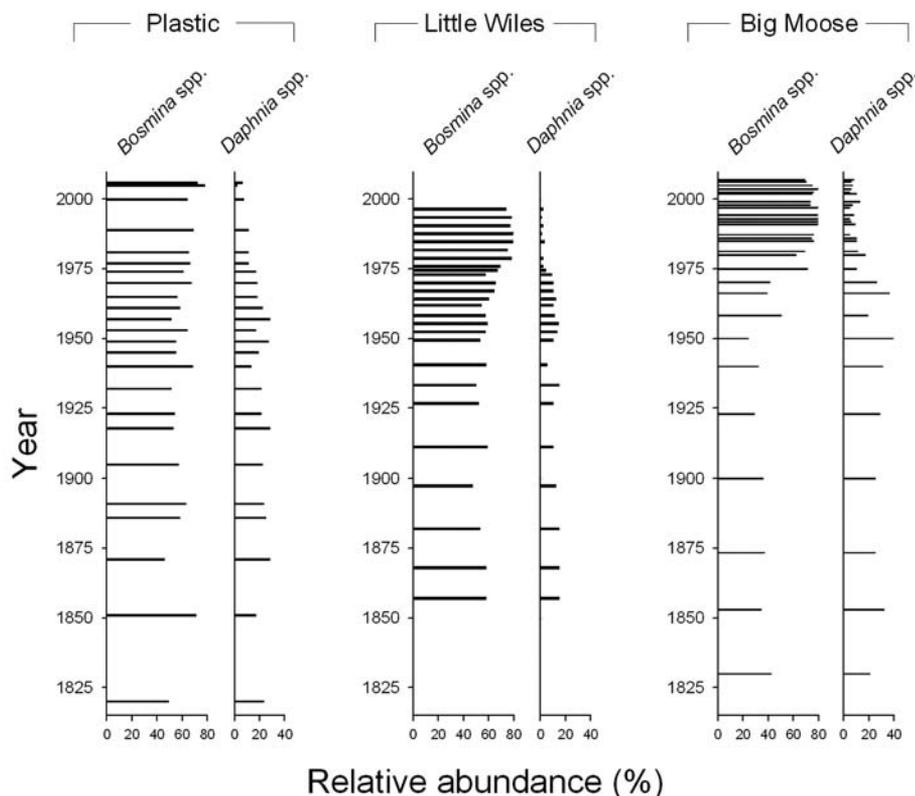
Fig. 3. Cladoceran relative abundances over time from lake sediment cores of three geographically distant North American lakes. Changes in the relative abundance of the two dominant pelagic cladoceran zooplankton groups (calcium-poor *Bosmina* spp. and calcium-rich *Daphnia* spp.) among sedimentary zooplankton assemblages from Plastic Lake (Ontario, Canada), Little Wiles Lake (Nova Scotia, Canada), and Big Moose Lake (New York, U.S.) are shown. The *y* axis denotes sediment age as estimated by ^{210}Pb analysis.
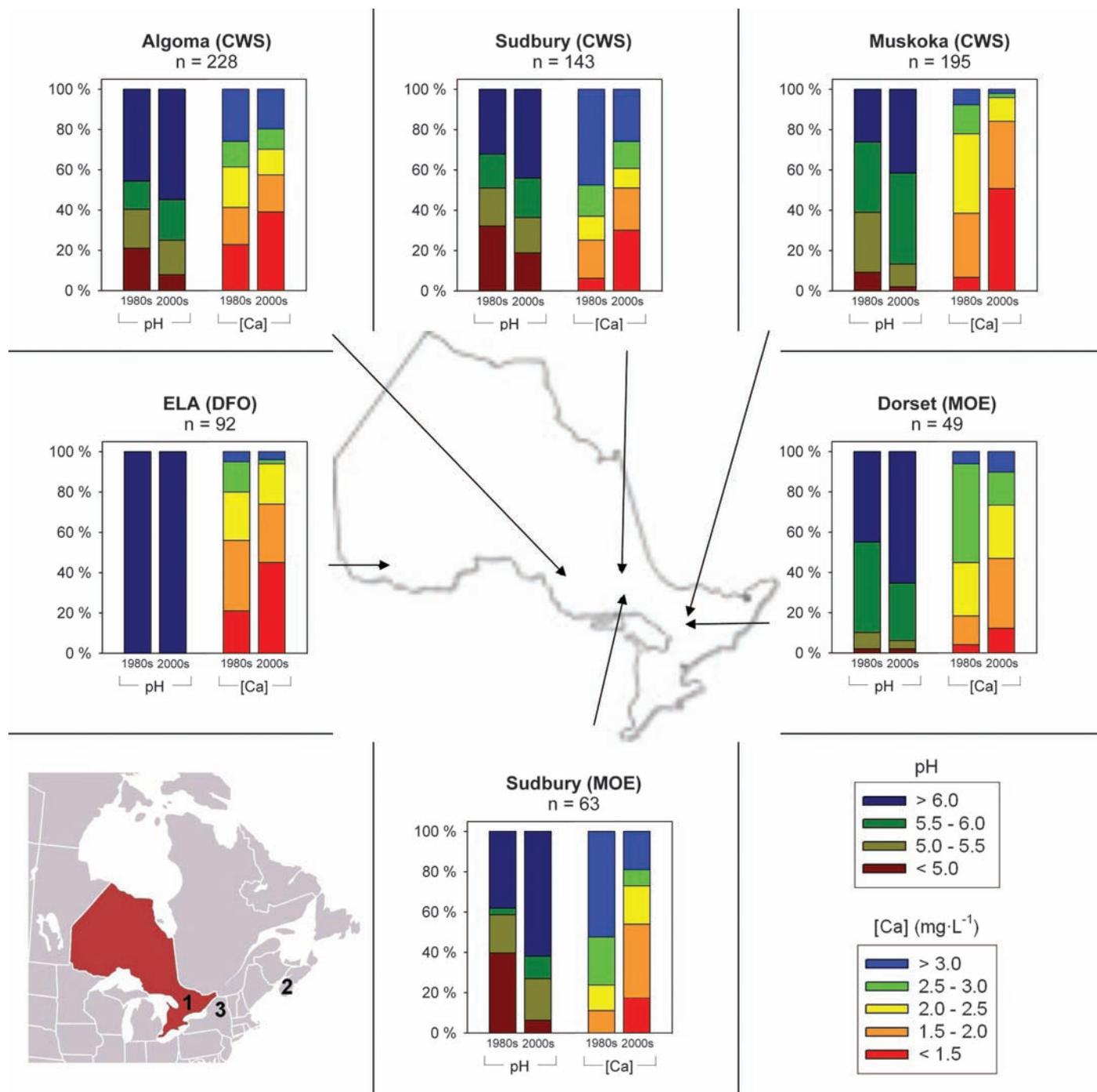
Fig. 4. Regional changes in lake-water pH and calcium concentration across Ontario, Canada. The map shows changes in the proportion of lakes by broad pH and calcium categories between the 1980s and 2000s for 770 lakes (see supporting online material text) spread across six study regions of Ontario, Canada. Data sets include: Dorset (Ontario Ministry of the Environment); Sudbury (Ontario Ministry of the Environment); Experimental Lakes Area (ELA; Fisheries and Oceans, Canada); and Algoma, Muskoka, and Sudbury surveys that were conducted by the Canadian Wildlife Service (CWS). (Inset at Bottom Left) Location of Ontario within eastern North America and locations of Plastic (1), Little Wiles (2), and Big Moose (3) lakes. n, number of lakes in subset; DFO, Fisheries and Oceans, Canada; MOE, Ontario Ministry of the Environment.

over their smaller, less visible, calcium-poor competitors (25, 27, 28). We did see increases in daphniids in our survey, perhaps driven by these trends, but only in those lakes with calcium concentrations >2.5 mg L^{-1} (Fig. 2B); when calcium concentrations were lower, daphniid populations fell.

It remains unclear what proportion of European and northeastern North American lakes has fallen below 1.5 mg L^{-1} calcium. We have examined the extent of calcium decline throughout Ontario using several long-term monitoring data sets (Fig. 4). Currently, 35% (12 to 51% among regions) of the 770 lakes have calcium concen-

trations <1.5 mg L^{-1}, and 62% (47 to 81%) are below 2.0 mg L^{-1} (particularly those among the small lakes sampled by the Canadian Wildlife Service). It is also apparent that calcium decline is occurring in lakes with relatively low acid inputs—for instance, in the Experimental Lakes Area of northwestern Ontario, Canada (Fig. 4).

Aqueous calcium concentrations are already either below or near experimentally defined thresholds of population fitness for calcium-rich crustacean zooplankton in a large proportion of lakes on the southeastern Canadian Shield, and additional declines are predicted for the next half century (29). The declining calcium trend we have observed is not restricted to Ontario; similar patterns have been observed in many other softwater regions of Europe and North America (1–3, 30). Thus, we predict a similar threat to the abundances of calcium-rich zooplankton in other lake districts with historically high acid-deposition rates. Calcium-rich daphniids are some of the most abundant zooplankton in many lake systems, and their loss will substantially affect food webs. Furthermore, it is likely that the calcium decline will influence other aquatic biota, not just daphniids. The ecological effects may transcend aquatic boundaries to affect a variety of calcium-rich biota.

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Genomic Analysis of the Clonal Origins of Relapsed Acute Lymphoblastic Leukemia

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Most children with acute lymphoblastic leukemia (ALL) can be cured, but the prognosis is dismal for the minority of patients who relapse after treatment. To explore the genetic basis of relapse, we performed genome-wide DNA copy number analyses on matched diagnosis and relapse samples from 61 pediatric patients with ALL. The diagnosis and relapse samples typically showed different patterns of genomic copy number abnormalities (CNAs), with the CNAs acquired at relapse preferentially affecting genes implicated in cell cycle regulation and B cell development. Most relapse samples lacked some of the CNAs present at diagnosis, which suggests that the cells responsible for relapse are ancestral to the primary leukemia cells. Backtracking studies revealed that cells corresponding to the relapse clone were often present as minor subpopulations at diagnosis. These data suggest that genomic abnormalities contributing to ALL relapse are selected for during treatment, and they point to new targets for therapeutic intervention.

Despite cure rates for pediatric ALL exceeding 80% (1), treatment failure remains a formidable problem. Relapsed ALL ranks as the fourth most common childhood malignancy and has an overall survival rate of only 30% (2, 3). Important biological and clinical differences have been identified between diagnostic and relapsed leukemic cells, including the acquisition of new chromosomal abnormalities, gene mutations, and reduced responsiveness to chemotherapeutic agents (4–7). However, many questions remain about the molecular abnormalities responsible for relapse, as well as the relationship between the cells giving rise to the primary and recurrent leukemias in individual patients.

Single-nucleotide polymorphism (SNP) arrays have enabled genome-wide analyses of DNA CNAs and loss of heterozygosity (LOH) in cancer and have provided important insights into the pathogenesis of newly diagnosed ALL. We previously reported multiple recurring somatic CNAs in genes encoding transcription factors, cell cycle regulators, apoptosis mediators, lymphoid signaling molecules, and drug receptors in B-progenitor and T-lineage ALL (B-ALL and T-ALL) (8, 9). To gain insights into the molecular lesions responsible for ALL relapse, we have now performed genome-wide CNA and LOH analyses on matched diagnostic and relapse bone marrow samples from 61 pediatric ALL patients (table S1). These samples included 47 B-ALL and 14 T-ALL cases (10). Samples were flow-sorted to ensure at least 80% tumor cell purity before DNA extraction (fig. S1). DNA copy number and LOH data were obtained using Affymetrix SNP 6.0 (47 diagnosis-relapse pairs) or 500K arrays (14 pairs). Remission bone marrow samples were also analyzed for 48 patients (table S1).

These analyses identified a mean of 10.8 somatic CNAs per B-ALL case and 7.1 CNAs per T-ALL case at diagnosis (table S4 and figs. S2 and S4). Of the B-ALL cases at diagnosis, 48.9% had CNAs in genes known to regulate B-lymphoid development, including PAX5 (N = 12), IKZF1 (N = 12), EBF1 (N = 2), and RAG1/2 (N = 2) (tables S5, S6, and S9). Deletion of CDKN2A/B was present in 36.2% of B-ALL and 71.4% T-ALL cases, and deletion

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