



100 Years of Chalky Teeth Research: From Pioneering Histopathology to Social Good

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One hundred years ago, histopathology pioneer Bernhard Gottlieb described developmentally disrupted teeth as having “chalky enamel” and “chalky spots” that “crumble” easily. He also asked pivotal questions about the pathogenesis of “enamel hypoplasia” that remained enigmatic for almost a century. Today, breakthrough pathomechanistic investigations of chalky enamel are revealing surprising answers, and an allied translational initiative—The D3 Group for developmental dental defects (“D3s”)—is converting such scientific knowledge into social good surrounding prevention of tooth decay. Molar hypomineralisation (MH) affects 1-in-5 children worldwide and is well-evidenced, but poorly recognised, as a principal risk factor for childhood tooth decay. Given MH is causally linked to infantile illness, an exciting corollary is that medical prevention would lead to substantial reductions in decay. Here we reflect on the past century of chalky teeth research and retrace the path leading to recognition of MH as a global health concern. Five research eras, today’s four major D3s, and diverse experimental attacks are outlined alongside translational wins that have benefitted global health. Addressing hopes for medical prevention of MH, this centennial year’s pathomechanistic discovery is contextualised against past accomplishments and new opportunities. Finally, we note the translational value of accessible infographics for guiding future work, and forecast exciting prospects for the next century.

Keywords: global health, paediatric disorders, dental defects, dental caries, medical prevention, developmental biomarkers, serum albumin, biomineralization

100 YEARS IN A NUTSHELL

One hundred years ago, eminent medical scientist Bernhard Gottlieb described developmentally disrupted teeth as having “chalky enamel” and “chalky spots” that “crumble” easily (1, 2). He also asked pivotal questions about the pathogenesis of so-called “enamel hypoplasia” that remained enigmatic for almost a century – why are lesions scattered among normal enamel and do they reflect cellular or extracellular disruption? Today, breakthrough pathomechanistic investigations of chalky enamel are revealing surprising answers, and an allied translational initiative – The D3 Group (D3G) for developmental dental defects (“D3s”) – is converting such scientific knowledge into social good surrounding prevention of tooth decay (3–9)¹. The primary target of these

¹ www.thed3group.org/social-impact

efforts is molar hypomineralisation (MH), a developmental disorder of unknown cause (idiopathic) defined pathologically by discolored enamel lesions termed demarcated opacities. MH affects the back teeth (2-year molars, 6-year molars, or both)² of 1-in-5 children worldwide and is well-evidenced, but poorly recognised, as a principal risk factor for childhood tooth decay^{3,4}. Given MH is causally linked to infantile illness, an exciting corollary is that medical prevention would lead to substantial reductions in decay and dental caries – a worldwide blight incurring massive social and economic tolls⁵.

Here we reflect briefly on the past century of chalky teeth research and retrace the path leading to recognition of MH as a significant concern for global health. Five research eras, today's four major D3s, and diverse experimental attacks are outlined alongside translational wins that have brought social good worldwide. Addressing hopes for medical prevention of MH, the pathomechanistic breakthrough—fortuitously reported in this centennial year for chalky teeth—is contextualised against past accomplishments and new opportunities. Finally, we note the translational value of accessible infographics for guiding future work, and forecast exciting prospects for the next century.

THREE PIONEERS OF THE “ENAMEL HYPOPLASIA” RESEARCH ERA

“Chalky teeth” sound like a dental matter. But actually they may be the fossilised legacy of paediatric medical problems, as is the case with MH. This was realised by Gottlieb who is considered a founder of oral biology having instigated the field of oral histopathology during his medical training. His 1920 investigation of enamel defects in children overwhelmed by various contemporary diseases (e.g., rickets, syphilis, gastroenteritis, measles) led to the conclusion that injury of enamel-forming cells (ameloblasts) was secondary to defective calcification of extracellular enamel (1, 2). Six decades later, more-nuanced studies of infantile sheep by the pioneer of a later era—dentist-turned-researcher Grace Suckling—prompted the opposite conclusion, and the notion that enamel hypomineralisation results from primary injuries to ameloblasts became dogma until now (10–12). Gottlieb applied “chalky” descriptors both to immature enamel (as seen histologically) and to erupted teeth, as back then “enamel hypoplasia” embraced what today are known separately as hypoplasias (pits, grooves) and hypomineralised opacities.

Abbreviations: D3, developmental dental defect; MH, molar hypomineralisation; D3G, The D3 Group for developmental dental defects; AI, amelogenesis imperfecta; DDE, developmental defect of enamel; MIH, molar-incisor hypomineralisation.

²We use the classical terms 2-year/6-year/12-year molars (corresponding to second primary/first permanent/second permanent molars, respectively) because of the following translational benefits: (1) aetiologically, it is useful to align dental outcomes with the time course of medical onset in babies and young children; (2) clinically, and for early screening in particular, it is useful to align with tooth-eruption ages; (3) for public communication, these colloquial terms are logical and simple.

³www.thed3group.org/prevalence

⁴www.thed3group.org/health-risks

⁵www.chalkyteeth.org/prevention.html

Another early contributor to MH history was Joseph Turner, a dentist whose findings about “localized enamel hypoplasia” became memorialised with the term “Turner teeth.” In 1909 he reported that patches of discoloured enamel in permanent (adult) teeth often coincided with predecessor primary (baby, or deciduous)⁶ teeth that had been abscessed, prompting the conclusion that an abscess-related inflammatory process had earlier damaged the enamel organ of the developing adult tooth (13). Later investigators substantiated this idea and added traumatised baby teeth—usually intruded front teeth (incisors) in fallen toddlers—as a second means of disrupting enamel development locally in successional adult teeth (14). Hereafter we refer to these two types of ascribed disruption as local causes of demarcated opacities (as opposed to the idiopathic, systemic causation of MH).

Our third pioneer of the “enamel hypoplasia” era is physiologist May Mellanby who—together with husband Edward, a medical physiologist and discoverer of vitamin D—hypothesised that enamel defects associated with rickets reflect a nutritional insufficiency (15, 16). This led to a famous series of investigations that inspired global health improvements, as elaborated below.

FIVE RESEARCH ERAS

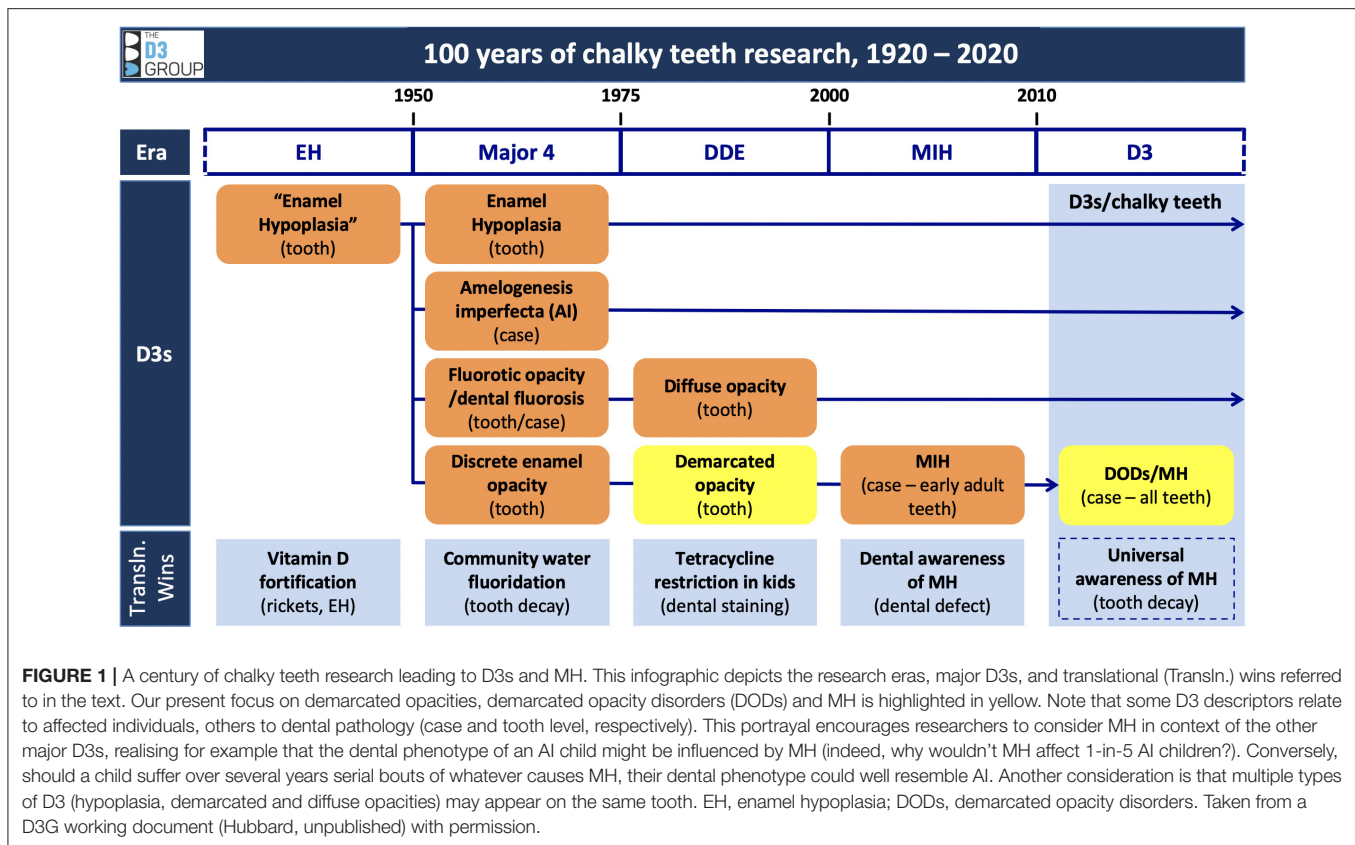
With hindsight, five research eras can be recognised as stepping stones to the present translational mix of D3s, chalky teeth and MH (Figure 1). The “enamel hypoplasia” era ended in 1949 with dentist Veikko Hurme's breakthrough report about “discrete enamel opacities,” later renamed demarcated opacities by Suckling (17, 18). This followed other investigators' delineation of fluoride-related opacities and amelogenesis imperfecta (AI) from true hypoplasia – so-called enamel pits and grooves (19, 20). Thus, the field now comprised four principal D3s, as holds today.

The “major 4” era, which ended with Suckling's emergence in the 1970s, featured fragmentary progress clouded by terminological indiscipline. Contrastingly, Suckling orchestrated a remarkably cohesive set of investigations that advanced pathomechanistic understanding greatly and produced a standardised clinico-scientific terminology—the developmental defects of enamel (DDE) index—that remains foundational to the D3 field (10, 18)⁷. The DDE era defined further hallmarks of MH, being idiopathic demarcated opacities in baby and adult teeth that are prone to degradation (so-called “post-eruptive breakdown”), plus a sporadic phenotype (affecting 1 to all 4 molars of any type) that seemingly contradicts systemic causation (21, 22). However, a case descriptor for the common clinical presentations of idiopathic demarcated opacities was lacking.

The “MIH” era commenced in 2001 when the term “molar-incisor hypomineralisation” (MIH) was introduced by European paediatric dentists to describe cases involving early

⁶We use the public-friendly terms “baby” and “adult” teeth to facilitate translational communication and also to align medical onset of MH (hypomineralised 2-year/6-year molars) with “sick babies”.

⁷www.thed3group.org/grace-suckling



adult teeth – namely 6-year molars, with or without co-affected incisors (23). Together with streamlining of the DDE index, a suite of pioneering studies by dentist-turned-researcher Birgitta Jalevik triggered numerous investigations ranging from epidemiology and clinical impacts to ultrastructure of chalky enamel. Detrimentially however, the restriction to early adult teeth sidelined 2- and 12-year molars—which, like 6-year molars, are the principal loci for childhood tooth decay⁴—and the inclusion of incisors raised scientific concerns about aetiological confounding. The latter issue reflects clinical inability to distinguish idiopathic demarcated opacities from those consequent to trauma or infection, which is an issue for all successive teeth – i.e., adult incisors, canines, and premolars (10, 24, 25).

The “D3” era started a decade later with a biochemical investigation of chalky enamel directed toward medical prevention of MH and allied decay, and launch of D3G’s translational education resource and public-facing “Chalky Teeth Campaign.” Being scientifically driven and prevention-centric, the ensuing D3 movement co-opted the term (idiopathic) MH to encompass the complete baby and adult dentitions and also avoid the aetiological complications surrounding adult incisors (3–5, 7–9, 26)^{8,9}.

⁸www.thed3group.org

⁹www.chalkyteeth.org

FOUR MAJOR D3S OF ENAMEL

Although demarcated opacities are center-stage of the MH story, it seems opportune to address their delineation (differential diagnosis) from the other 3 major D3s of enamel and also detangle some terminological confusion that frequents the literature. It is imperative that case descriptors (AI, dental fluorosis, MIH, MH) are distinguished from tooth-level pathology such as enamel opacities (Figure 1). For example, common misnomers such as “MIH molars” and “MIH enamel” overlook the fact that—consequent upon the sporadic phenotype—affected molars in MIH children usually contain both normal and abnormal enamel, and those children may also have partner molars that aren’t hypomineralised.

Based on historical understanding of causation and pathogenesis, major D3s have long been divided into primary groupings of genetic (AI) and acquired (the rest). AI comes in many flavors and is a key comparator for MH because, although rare, it can be devastating for affected families as most if not all teeth are disrupted (3). That some manifestations of AI feature demarcated opacities also holds aetiological interest for MH (27, 28). Diffuse opacities are a hallmark of fluoride overexposure (dental fluorosis) and usually can be distinguished from demarcated opacities through clinical appearance. However, as Suckling stressed, an individual tooth may have both demarcated and diffuse opacities and the latter may reflect causes other than fluoride. Distinguishing features

of demarcated opacities (distinct borders, discolouration, hard or chalky texture, acid lability) were reported by Hurme and then correlated to enamel cell biology by Suckling. Crucially, she linked diffuse and demarcated opacities to the secretory and hardening phases of enamel formation, respectively (10, 11, 17). Many investigators have since added a wealth of detail to these fundamentals, as follows.

A MULTITUDE OF EXPERIMENTAL APPROACHES AND ADAPTATIONS

The foregoing D3 learnings involved a battery of experimental approaches, many adapted from mainstream soft-tissue science to accommodate the technical challenges posed by dental enamel – our hardest tissue. To exemplify how MH studies have ranged from population level to nanoscale, it's notable that light microscopic histology as pioneered by Gottlieb remains informative today, boosted by modernisations including polarised light microscopy, immunofluorescence, scanning and transmission electron microscopy (11, 29–32). Biophysical investigations have grown from Hurme's simplistic acid dissolution of chalky spots to advanced analyses of chemical composition and hardness (30, 33–35). With time, biochemical approaches were used to query pathogenesis of chalky enamel at protein level (26, 36–39). Together, these tooth-level analyses showed that chalky opacities exhibit elevated porosity and carbonate levels (i.e., more chalk-like), plus high amounts of protein consistent with incomplete hardening.

Animal models added pathophysiological insights, ranging from Mellanby's rachitic puppies and Suckling's gastroenteritic lambs through to toxicological and gene-knockout studies in rats and mice (10, 15, 40, 41). Further understanding came from human population and genetics studies (epidemiology, twins, single nucleotide polymorphisms), which together reinforced longstanding belief that idiopathic demarcated opacities (and so MH) are primarily of acquired/non-genetic origin (17, 42–46). As such, this bodes well for medical preventability of MH – notably, even partial alleviation of severe phenotypes (i.e., hard-white, rather than chalky opacities) would normalise risk of decay⁴. However, as with other acquired conditions, we must expect that complex genetics may also influence outcomes to some degree.

FIVE TRANSLATIONAL SUCCESSES

It seems remarkable (indeed inspirational) that such a boutique field as D3s has inspired a string of translational successes, two of which have benefitted global health through integration of medicine and dentistry (Figure 1). While common practice today, vitamin D-balanced lifestyles trace back to the Mellanbys' discovery that cod liver oil cured rickets in dogs – one manifestation of which was “enamel hypoplasia” (15, 16). These findings were amplified and then translated to population level through vitamin D-rich foods, leading to containment of rickets and consequent improvements to dental health—namely less hypoplasia and allied decay—by the 1940s (47). A second medico-dental win followed introduction of tetracycline

antibiotics in the early 1950s. While tetracyclines provided a magic bullet for many paediatric infections, unsightly grey/brown staining of tooth enamel was an unacceptable side-effect. The causal link to tooth development was soon recognised and medical restriction of tetracycline use to people older than 8 years was implemented worldwide over the course of 2 decades. The translational success of community water fluoridation—another nutritional intervention that led to global reductions in tooth decay—needs no elaboration other than to note progression from initial clinical observations to successful management at population level took several decades (3).

More modestly, the MIH era successfully translated problem-awareness (and allied clinical guidelines) from a select few researchers and practitioners out to the wider dental community¹⁰. The D3 movement in turn is extending awareness of MH and the all-important connection to childhood tooth decay more broadly across the healthcare sector and society. By publicising MH as a medically-acquired disorder, this prevention-centric initiative is using a medico-dental platform to argue for well-funded cross-disciplinary research (3)⁵.

THREE DS AND A SOCIAL-GOOD MOVEMENT DRIVEN BY SCIENCE

It struck the first author that, if the real world was to get a grip on the 3-layered “MH problem” —comprising MH itself, allied educational gaps, and lack of scientific understanding—there would need to be an accessible language embedded within a cross-sector translational approach. Initially this inspired coining of “D3” as an abstract substitute for the technical tongue-twister it stands for (3). D3G has since developed a “translational lingo” (e.g., “chalky teeth,” “chalky molars,” “Molar Hypomin”) combining clinico-scientific and public faces, and mined the historical record to put “chalky terminology” on a solid scientific and social footing^{11,12}. These and other social-good aspects of the D3 movement reflect D3G's philanthropic origins, whereas the scientific “pointy end” draws on career scientists working collaboratively with practitioners and other stakeholders. Unique outcomes of this scientifically-driven translational approach include an international cross-sector network embodying numerous researchers of diverse ilk (“D3G's research army”), a “one-stop” online-education resource featuring tools for researchers and affected children alike, and a nascent practice-based research network (3, 9)^{8,13,14}.

D3G's aspiration to prevent MH through paediatric medical intervention reflects expertise of the biomedical research team at its core. They attacked this lofty goal by addressing some basic questions about chalky opacities, hoping that answers would eventually elevate idiopathic MH to a disorder of known causation as follows.

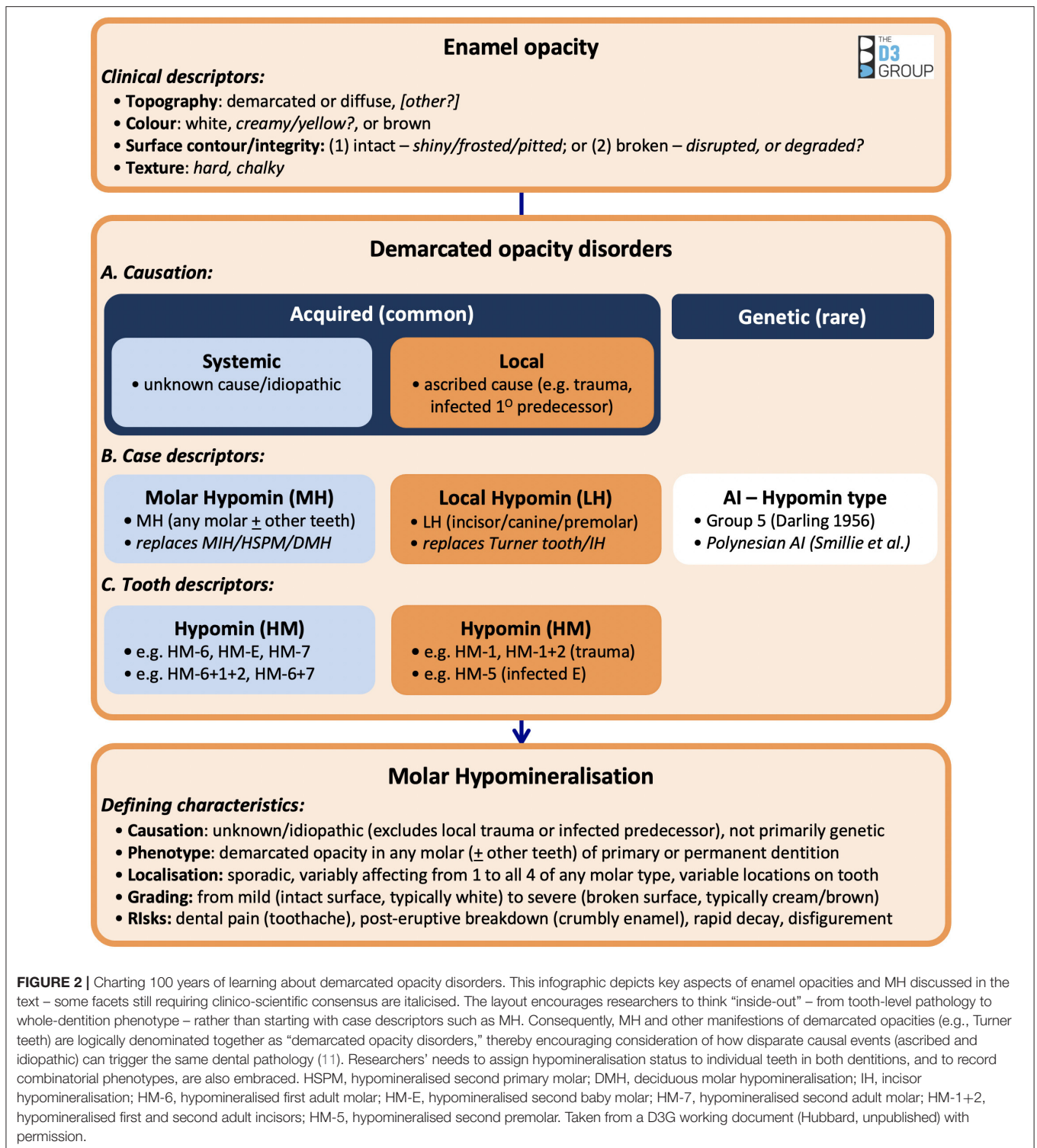
¹⁰ www.thed3group.org/specialist-4

¹¹ www.thed3group.org/what-are-chalky-teeth

¹² www.chalkyteeth.org/speak-chalky-teeth.html

¹³ www.thed3group.org/international

¹⁴ www.chalkyteeth.org/we-fight-chalky-teeth-practices.html



ONE UNEXPECTED PROTEIN AND A 100-YEAR BREAKTHROUGH

Gottlieb’s century-old questions about opacity localisation within enamel, together with Suckling’s intrigue over 6-year molars

being affected sporadically, led dentally-trained biochemist Mike Hubbard to reason that MH pathogenesis involves 2 mechanistic levels: (1) a local (dental) pathomechanism responsible for chalky enamel; and (2) a systemic (medical) disruption that triggers the localised dental event. Using proteomics and an

inside-out strategy to address the local pathomechanism, chalky enamel was found to contain numerous proteins when the opacity surface was visibly broken, but only one prevalent protein if the surface layer was intact and so impervious to oral fluid (26). That the latter “fossilised protein” was serum albumin—and not the principal enamel protein amelogenin—was unexpected given long-established connections between retained amelogenin and defective enamel (37, 38). Such presence of albumin also had to be treated cautiously given a contentious history concerning experimental artifact (26, 39, 48).

Two follow-up studies in 2020 overcame the artifact concerns and addressed medical-onset and dental-outcome perspectives, respectively. The collective biochemical findings provided cohesive evidence that the local pathomechanism does indeed involve exposure of unhardened enamel to serum albumin (7, 8). This discovery, plus evidence that albumin resists the proteolytic attack normally used to remove amelogenin—thereby enabling enamel to harden—provides breakthrough insight to the pathogenesis of chalky enamel. The ensuing pathomechanistic model, termed “mineralisation poisoning,” involves persistent blockage of enamel hardening by albumin and plausibly accounts for the clinical characteristics of demarcated opacities and MH. It also answers Gottlieb’s question by establishing that, at its ultimate crux, MH involves direct disruption of immature enamel and not injury to enamel cells as held by dogma – the latter may well be involved in penultimate pathologic steps however. This fundamental advance holds significance for future clinical management and opens an exciting research avenue toward medical prevention of MH (6–8).

SEVERAL INFOGRAPHICS AND A SYMPOSIUM TO GUIDE FUTURE RESEARCH AND LEARNING

If the scientific path to victory over MH is to be smoothed, shortcomings exemplified by today’s muddled literature must be addressed with a focus on standardisation (e.g., common terminology and definitions extending across laboratory and clinical practice). This isn’t to say that off-track activities should be stifled, rather that a strong consensus should be identified from which to base future thinking and communication. We hope that by condensing a century of learning into a series of accessible infographics (e.g., the working documents depicted in **Figures 1, 2**), the field will soon gain a consistent understanding of past achievements and then develop a robust framework for future work. Translationally, such educational tools should be dynamic, readily available online, and adaptable to various audiences. Cross-disciplinary dialogue will be required to achieve consensus and marry the demands of scientific method with the realities of clinical practice, as exemplified by proposed lab-friendly clinical descriptors italicised in **Figure 2**. Fittingly, the century is closing with D3G’s inaugural international workshop and symposium, which bring unprecedented opportunity to

involve all stakeholders in such discourse pertaining to MH and chalky teeth¹⁵.

SOME CLOSING THOUGHTS ABOUT THE NEXT CENTURY

The past century has seen chalky teeth research grow from pioneering histology into a comprehensive international endeavor framed around science translation and social good. Gottlieb’s “chalky spots” have been illuminated in molecular detail, providing pathomechanistic insight to his visionary intrigue about teeth erupting with isolated patches of crumbly enamel. Strikingly, the field now has protein biomarkers with aetiological and clinical potential, spurring exciting thoughts about digital dentistry workflows connecting seamlessly to laboratory and epidemiological investigations. The emergent handle on MH pathogenesis opens the door to concerted trans-disciplinary attack on medical prevention, bolstered by integrated educational and advocacy programs. If translational-research cards are played well, coming years may see funders hunting for D3 researchers—rather than the opposite situation we all suffer today—and timescales for translation to social good may decrease from decades to years. We close by tipping our hats to the multitude of investigators who elevated the D3 field to what it is today, and encourage researchers of all station to consider adding “translational D3ology” to their future interests.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MH conceived, drafted, and critically revised the manuscript. VP and BG made underpinning intellectual contributions and critical revisions. All authors contributed to the article and approved the submitted version.

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¹⁵www.thed3group.org/toronto2020

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Conflict of Interest: MH is founder/director of The D3 Group for developmental dental defects (www.thed3group.org), a charitable network.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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